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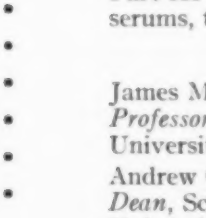

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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

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The Pharmaceutic Utilization of Materials Derived from Human Erythrocytes*

By Robert D. Barnard†

By the end of 1943 over one million volunteer blood donors had each given one or more pints of blood for use by the armed forces. During that year the energies of the technical staffs of the agencies concerned were directed toward the perfection of economical collection and efficient plasma processing techniques. Little attention was given to the possibilities of that 50 per cent of whole blood not as yet widely utilized in the treatment of war injuries but which represents, nevertheless, a wealth of material upon whose salvage the ultimate fate of plasma banking may depend.

Some portion of the failure to carry out an intensive program aimed at the utilization of such a wealth of species specific protein as is represented by the erythrocytes discarded from plasma processing undoubtedly could be attributed to the uncertainty as to the future of the plasma program. While this uncertainty has not entirely disappeared it seems probable that extensive, if not large-scale, blood banking is here to stay.

In 1936, the first blood bank in this country was set up at the Cook County Hospital by the late Dr. Bernard Fantus, who saw clearly the direction of the evolution of the institution. At the time of the founding of the blood bank, he directed that studies aimed at the utilization of waste human corpuscles be undertaken and he, himself, outlined the nature of these studies. With respect to his foresight, it may be said that there has been no departure in blood banking or in its subsequent development which was not original with Dr. Fantus. The outlines of the directions such work was to take were drawn up in 1936 and have been modified only in slight detail as work progressed along with concomitant advances in our knowledge of pharmaceutic and blood pigment chemistry.

EXPERIMENTAL

The present report consists of a review of the work started in 1936 (and carried through until the fall of 1940) on the fractionation of human erythrocyte residues from the blood bank at the Cook County Hospital. It outlines the principles of these fractionations, lists and describes some of the products prepared and indicates possible pharmaceutic uses to which the fractions obtained may be put. It is important to emphasize that little clini-

* Received Nov. 29, 1944, from the Division of Medical Research, Department of Medicine, Ohio State University, Columbus, Ohio.

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cal application has as yet been made of these fractions, the majority of which have either not been previously described in the literature or have received mention only in other than pharmaceutic connection.

In many instances the application of the prepared fractions to therapeutics is purely speculative in the sense that no experimental work has been done on them in this connection, the presumption of their application being arrived at because of their physical or biochemical properties. In other instances there seems to be adequate clinical or experimental evidence of such application. The latter instance will be indicated during the course of the report.

In Fig. 1 there is presented the breakdown scheme in human blood processing. Our concern here is exclusively with the erythrocytes. Human erythrocytes separate very readily from citrated or heparinized blood and less readily from the dextrose fortified blood used in whole blood preservation. After the plasma has been used for its own processing, the remaining cells are washed twice with 0.9% NaCl solution. Sedimentation is not nearly so rapid in this instance so that centrifugation is usually necessary. This should be carried only to the point where the leucocytes remain in suspension and can be removed along with the wash saline. Human red cell suspensions made by a third restoration of the original blood volume with fresh, CO_2 saturated saline have been employed (*a*) by transfusion in anemias (1) and may be indicated in methemoglobinemia and in carbon monoxide or isonitrile poisoning. Other concentrations of erythrocyte suspension have been used (*b*) as specific hemagglutinogen for the removal by adsorption of the type specific hemagglutinin of plasma or serum designed for massive intravenous infusion or of tissues for grafting (2), (*c*) as indicator in complement fixation tests based on human hemolytic systems such as the Noguchi test, and (*d*) as an antigen in the Bordet medico-legal test for human blood. Erythrocyte paste has been used as a dressing for wounds (3).

1A. Stroma Gel.—The toluenized stroma substance is removed from the surface of the cell protein solution and transferred to a closed system where the toluene is removed by vacuum distillation. This leaves a creamy-white paste which turns red on exposure to air and consists mainly of free lecithin and cholesterol lecithitide. As such, it may find usage by intravenous injection in the treatment of systemic poisoning by arsine and the substituted arsines (lewisite), all derivatives of which are quite soluble in corpuscular stroma; even that of the intact erythrocyte. It is by virtue of that solution in the stroma that these substances act as hemolytic poisons. Infusions of whole blood or corpuscle suspension have been used in the treatment of this type of poisoning but have been found to be unsuitable because the additional hemolysis produced by the extra erythrocytes in the circulation has resulted in massive hemoglobinuria and death from renal insufficiency. The use of stroma substance alone would be free from this objection and should be tried experimentally not only in poisoning by the arsines but also in those caused by other chemical hemolytic agents such as phosphine, aniline and pyrogallol as well as in infections by certain hemolytic viruses [yellow fever; blackwater (?)].[1]

1B. Oxyhemoglobin Solution.—The material left after removal of the stroma gel consists of an approximately 5 millimolar (8%) oxyhemoglobin solution containing a small amount of cell globulins. (These are precipitated almost completely by fur-

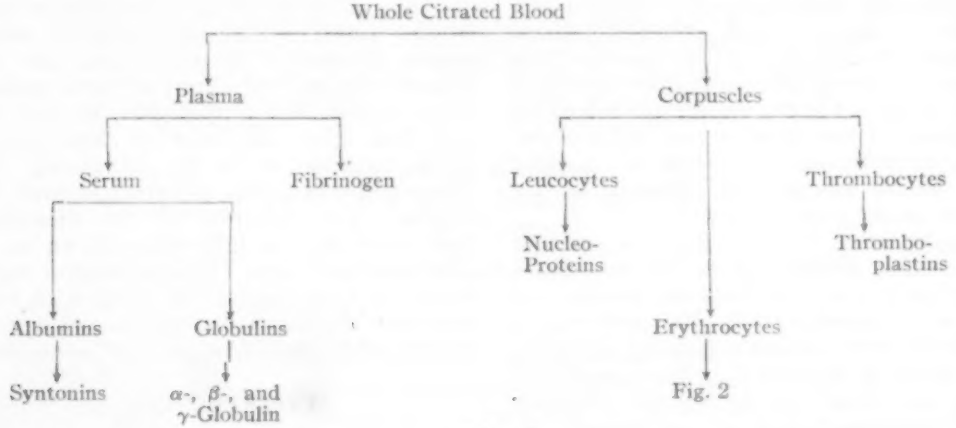

Fig. 1

The fractionation of erythrocytes is shown in Fig. 2. It is effected by treating one volume of corpuscle paste with three volumes of distilled water and one-half volume of toluene (4) and then shaking vigorously. The corpuscles are laked and the stroma rises to the surface along with the ex-

[1] Stroma substance obtained by high-speed centrifugation and mechanical disruption of the erythrocytes has recently been used by Wiener (5) as an absorbent for anti-Rh factor and type specific hemagglutinins.

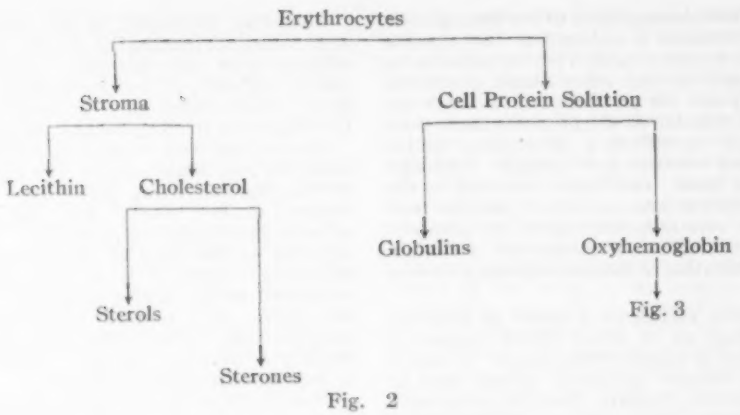

Fig. 2

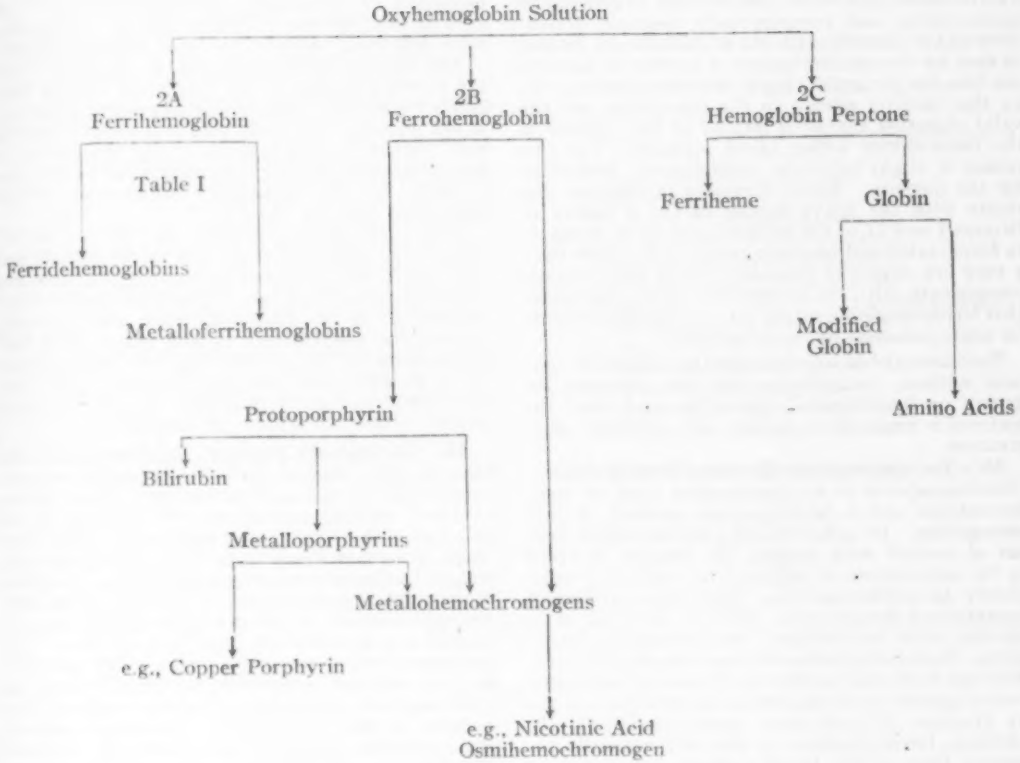

Fig. 3

ther dilution, although, as has been stated above, the removal of cell globulin is not practiced.) It is about this concentration which has been employed by Amberson (6) as a suitable osmotically active colloid for plasma substitution. For this purpose, the trace of remaining toluene which has so far acted to inhibit putrefaction must now be removed. This removal is effected by suction evaporation of water until the solution is about two-thirds of its original volume, or until no toluene is detectable therein. The original volume is then restored by the addition of water.

Five millimolar oxyhemoglobin is a convenient concentration of the pigment for use in the preparation of all other derivatives to be described. These may be divided into four major categories: those of 2A, ferrihemoglobin; 2B, ferrohemoglobin; 2C, hemoglobin peptone; and 2D, porphyrins. The relationship of these derivatives is shown in Fig. 3.

2A. Ferrihemoglobin.—By the addition of 1 equivalent of alkali ferricyanide, undenaturated oxyhemoglobin in solution is converted completely and almost instantaneously to ferrihemoglobin (commonly termed methemoglobin). Other oxidizing agents promote the same change but almost invariably with an accompanying denaturation of the globin portion of the molecule. During the course of the oxidation of oxyhemoglobin (through the

intermediary of ferrohemoglobin) to ferrihemoglobin, the added ferricyanide is reduced to ferrocyanide and the latter is not removable from the solution by subsequent dialysis or any other simple expedient. Its presence appears not to affect the utility of the ferrihemoglobin solution or the products made from it, but it might introduce a theoretic objection to the use of such solutions parenterally. Although these objections would seem to be overruled by the relative nontoxicity of ferri- and ferro-cyanides, pure ferrihemoglobin solutions may easily be prepared without the introduction of extraneous materials, by the anodic oxidation of ferrohemoglobin solutions (7).

Ferrihemoglobin undergoes a series of clear-cut chemical reactions all of which afford suggestion for its possible use in human therapeutics. It reacts with low ionic volume hydroacid anions such as cyanide, cyanamide, cyanate, fluoride, fulminate, azide, thiocyanate, hydrosulfide, hydroperoxide hydroselenide and nitrite (as nitro ion) to give rise to undissociated and comparatively nontoxic derivatives (3A). Ferrihemoglobin is deliberately formed *in vivo*, by the administration of nitrites or methylene blue for the antidoting of cyanide poisoning (8). In this case its advent in the circulation has the valid objection that it is formed at the expense of the respiratorily active blood pigment. For this reason it might better be administered, preformed for the purpose. Ferrihemoglobin in solution also reacts with the heavy metals of the *b* family of Groups I and II of the periodic system of elements to form stable and nontoxic compounds which have a very low degree of dissociation into their original components (3B). It is therefore not inconceivable that ferrihemoglobin might act as a specific antidote for acute poisoning by these elements.

Ferrihemoglobin solutions may be reduced to dryness without denaturation by the common ice drying or lyophilization procedure and thus the material is amenable to storage and indefinite preservation.

2B. Ferrohemoglobin (Reduced Hemoglobin).—Ferrohemoglobin is an electronated form of ferrihemoglobin and a deoxygenation product of oxyhemoglobin. Its solutions are unstable unless kept out of contact with oxygen, the tension of which in the atmosphere is sufficient to convert it completely to oxyhemoglobin. The latter undergoes spontaneous denaturation either in solution or in the dry state to ill-defined ferrihemoglobin conjugates. Ferrohemoglobin solution when kept oxygen-free may be stored indefinitely without denaturation even if grossly contaminated, a fact first pointed out by Gamgee (9) and since amply confirmed. In addition, ferrihemoglobin is the only naturally occurring form of the blood pigment which can be dried without loss of its property of combining reversibly with molecular oxygen on subsequent resolution (10, 11).

Other than for the implications presented by the last fact, little consideration has been given to the possible uses of ferrohemoglobin in human therapeutics. Solutions of the material, when injected into the circulation, might have some slight theoretic advantage over oxyhemoglobin solutions in the treatment of carbon monoxide poisoning. The technical difficulties of the preparation and administration of the pigment in this form, however, would offset the advantage over oxyhemoglobin solutions, which in any event are converted to ferrohemoglobin in the capillaries of the systemic circulation.

Following the report of the absorption of iron after intestinal instillation of whole blood to human subjects with hypochromatic anemia (12), enteric coated capsules of ferrohemoglobin powder have been recommended as a possible hematinic (13). Its efficacy for the purpose has not been learned.

Coextensive with experiments devised to supplement the iron intake with enteric coated capsules of ferrohemoglobin, have been those in which dried human oxyhemoglobin has been administered without enteric coating protection. The author has objected to the merit of the procedure since oxyhemoglobin (even if it has not been completely denatured by the drying process) is acted upon by the gastric juice to form the insoluble ferriheme chloride (*vide infra;* hemoglobin peptone), the ferric iron of which appears to be not available, at least on enteral administration.

More recently, the administration of ordinary capsules of "defatted" crude ferrohemoglobin as a hematinic has been under consideration (14). The iron in this pigment is ferrous and an acid gastric juice will react with it to form ferrous chloride.

The iron in ferrohemoglobin is combined with the pyrrol nitrogens of the porphyrin ring by ionic bonds exclusively (15). For that reason, treatment of the pigment with weak acid will remove the iron and furnish protoporphyrin. The treatment of ferrohemoglobin solutions by acid is the basis of two methods for the laboratory preparation of the fluorescent pigment (16, 17).[1]

While protoporphyrin and the derivatives obtainable directly from it are not species specific and can therefore be prepared for any purpose from any vertebrate blood (the same obtains for the ferriheme derivatives to be described) they will be incorporated for completeness in this report since their preparation fits with facility into the entire scheme of the prosecution at the same time and in the same laboratory as the preparation of the distinctly species specific blood pigment derivatives.

2C. Hemoglobin Peptone, Ferriheme and Globin.—In this category, there is lumped for convenience in classification, all derivatives of hemoglobin obtained through proteolysis whether this be the result of ionic or enzymatic hydrolysis. The initial stage of such cleavage is to yield a low molecular weight ferriheme derivative and globin. Ferriheme is the starting point for the preparation of the ferrihemochromogens, to be discussed further on, and as such it is an extremely important chemical. Unfortunately its preparation in reasonably pure form and in sufficient quantities for pharmaceutic use is an expensive procedure calling for relatively large volumes of glacial acetic acid. In order to provide the enormous quantities of crystalline ferriheme chloride (hemin) that may ultimately be essential, the need for suitable methods for the preparation of this substance is a pressing one. Ferriheme chloride, until now a laboratory reagent of very limited application [viz., in the preparation of hemoglobinometry standards (18, 19), a color reagent for reducing sugars (20), and a precipitant for certain alkaloids] (7) may develop extensive utility since it enhances the effect of certain endocrine products (21, 22) and is being used in the preparation of at least one experimental "depot" insulin product (23). It is

[1] Since the elaboration of the latter method for the preparation of protoporphyrin (17) one of even greater expediency has been developed. Dry ferrohemoglobin, when treated in anhydrous alcohol suspension with hydrogen chloride, yields a pure protoporphyrin hydrochloride solution.

unlikely that the future human globin production to meet pharmaceutic needs will furnish adequate supplies of ferriheme from this source since globin makes up 96%, whereas heme only the remaining 4%, by weight, of the hemoglobin molecule. More likely the waste erythrocyte paste of the packing industry will be exploited. Simple acid hydrolysis of ferrihemoglobin (indirectly, also, of oxyhemoglobin) yields crude ferriheme and globin. It has been shown recently that orally administered protein hydrolysates are effective in the correction of hypoproteinemia (24). Globin is not a biologically complete protein, but its hydrolysates are used for the supplementation of other protein cleavage mixtures to its essential amino acid histidine. Ferriheme residues from this source are as yet a potentiality, only. Globin, from animal sources, in human therapeutics does not offer much promise of exploitation since, theoretically at least, they do not offer the advantage of nonallergenicity for human beings afforded by human globin.

The last is already employed extensively in the preparation of a "depot" form of insulin (Globin insulin BW & Co.) and experimentally in modified form as an oncotically active colloid for blood substitution (25) and as a tanning agent in the plasma or serum exudative skin conditions (7) (second or third degree burns; the fibrinorrheic dermatidites).

2D. Protoporphyrin.—Protoporphyrin may be prepared most conveniently from dried ferrohemoglobin (2B). Protoporphyrin (hemoporphyrin) solution has been advocated as a therapeutic agent by European authors but has had little application in this country. Its solutions and those of certain of its modifications have a brilliant red fluorescence, and were the material sufficiently plentiful it might find application in commerce or the arts.

While there are no anemias on record which have as their basis the failure of protoporphyrin synthesis, the effect of porphyrin on certain obscure anemias might be worthy of trial. Other derivatives of porphyrin which may be utilizable therapeutically are listed later in this report along with other nonspecific blood pigment derivatives whose preparation from nonhuman blood sources may be more advantageous.

In Table I, the known derivatives of ferrihemoglobin are tabulated. These fall into two categories, 3A, the ferridehemoglobins and 3B, the metalloferrihemoglobins. A tautomer of ferrihemoglobin is also thought to exist (7).

(also in some instances with ferriheme or the ferrihemochromogens). The ferridehemoglobins are spectroscopic entities and are much more stable in solution than is ferrihemoglobin itself. Since the pharmacodynamic action of the hydro-acid anion is curtailed by ferrideheme formation (probably the manner in which they exert their pharmacodynamic effect) the ferridehemoglobins are not toxic. Whether they should present any distinct advantage over the unconjugated pigment as osmotically active colloids, has not been put to experimental trial. The existence of a ferrihemoglobin thiocyanate taken in conjunction with the reputed efficacy of the anion in the treatment of spastic hypertension suggests the trial of the ferridehemoglobin in that connection while the cyanamide derivative, because of the peripheral vasodilator properties of the anion, suggests itself in the experimental therapy of those conditions where peripheral vasodepressor activity might be desirable as in Raynaud's disease and Buerger's disease.

TABLE I.—DERIVATIVES OF FERRIHEMOGLOBIN

	(3A) FERRIDEHEMOGLOBINS
(*a*)	Azide ferridehemoglobin
(*b*)	Cyanamide (carbimid) ferridehemoglobin
(*c*)	Cyanate ferridehemoglobin
(*d*)	Cyanide ferridehemoglobin
(*e*)	Fluoride ferridehemoglobin
(*f*)	Fulminate ferridehemoglobin
(*g*)	Hydroperoxide ferridehemoglobin
(*h*)	Hydroselenide ferridehemoglobin
(*i*)	Hydrosulfide ferridehemoglobin
(*j*)	Hydroxide (alkaline methemoglobin) ferridehemoglobin
(*k*)	Guanidine ferridehemoglobin
(*l*)	Creatinine ferridehemoglobin
(*m*)	Creatine ferridehemoglobin
(*n*)	o-Benzoic sulfinide ferridehemoglobin
(*o*)	Thiocyanate ferridehemoglobin
	(3B) METALLOFERRIHEMOGLOBINS
(*a*)	Copper ferrihemoglobin
(*b*)	Cadmium ferrihemoglobin
(*c*)	Zinc ferrihemoglobin
(*d*)	Mercury ferrihemoglobin
(*e*)	Silver ferrihemoglobin
(*f*)	Gold ferrihemoglobin

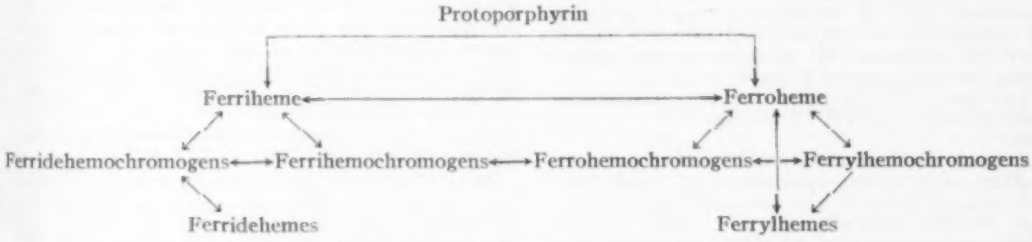

Fig. 4.—The Interrelationship of the Iron Porphyrin Derivatives.

3A. Ferridehemoglobins.—These are the reaction products of ferrihemoglobin with the low ionic volume hydro-acid anions previously enumerated, all of which, in their ionic form, are tissue asphyxiants (8) and all of which are rendered inert in this regard by their combination with ferrihemoglobin

3B. Metalloferrihemoglobins. Of the seven metalloferrihemoglobins (26) tabulated in Fig. 4 (in addition to those of the heavy metals, one with beryllium appears to exist), only the silver and mercury derivative have been prepared in any state of purity and have had any clinical application

which has been limited to their uses as a mild silver (27) and a mild mercury protein, respectively. In this connection, the derivatives of beef hemoglobin have been employed but more recently the human metalloferrihemoglobins are under investigation because of the possibility of their finding a place in parenteral therapy and the desire, in such usage, to circumvent possible allergenic complications. Zinc, copper and cadmium ferrihemoglobin have no conceivable use at the present time and, in fact, have not been prepared in a pure form.

4. The Hemochromogens (Fig. 4).—The iron porphyrin nucleus of hemoglobin or a radical almost identical with it, chemically, is ubiquitous in living substances. It is the prosthetic nucleus not only of the vertebrate (and some invertebrate) circulating respiratory pigment but also of the cytochromes, the internal respiratory enzymes of all cells. It is the essential portion of the proteinaceous molecule of such important enzymes such as catalase, peroxidase and possibly cholin-esterase, aminoxidase and many others. It is a constituent of certain pathologic pigments (malaria pigment; mottled enamel?). It is not strange that early attempts were made to utilize the substance itself, which may be prepared in crystalline form, in human therapeutics. Brown and Loevenhart (28) administered alkaline solutions of "hematin" intravenously with toxic effects; they, in fact attributed the paroxysms of malaria to a liberation of ferriheme from the parasitized erythrocyte. Warburg (29) showed that nicotine ferrihemochromogen acted *in vitro* as a respiratory catalyst, but no therapeutic application of this effect was considered. Pilocarpine (30), iminazolyl (31) and physostigmine (7) conjugates have been prepared. Fischer (32) synthesized ferriheme and established its structural formula, though the brilliant investigations of Willstätter (33) and Kuster (34) paved the way for this contribution.

Hirsch and Barnard (35) attempted the antidoting of cyanide poisoning in dogs by the intravenous administration of alkaline solutions of ferriheme. The attempt was based on the reaction between cyanide ion and ferriheme; it was believed that the weight ratio of molar ferriheme to ferrihemoglobin being 1:14, the former should be 14 times as effective as the latter as an antidote. The dogs survived the acute cyanide poisoning but developed embolic phenomena from the intravascular precipitation of amorphous ferriheme, the latter being insoluble when brought to the *p*H of the blood. Attempts were then directed toward the formation of a reactive ferrihemochromogen which would be soluble at physiologic *p*H. A ferrihemochromogen prepared by conjugation of ferriheme and nicotinic acid in anhydrous ammonia (36) yielded a suitable product which, however, proved unstable on storage.

Anderson (37) prepared sodium ferrihemate, stable in solution at *p*H 7.6 but this proved toxic to dogs.

Recently ferrihemochromogens made in aqueous medium with various components of the B complex group of vitamins and water soluble at physiologic *p*H have been prepared (38). Some of these ferrihemochromogens are excellent respiratory catalysts, some have cholinesterase characteristics while others are being developed as sources of iron, vitamin B components or both for parenteral administration in certain nutritional deficiency diseases.

In the same category with the hemochromogens are blood pigment derivatives, in which a quaternary nitrogen compound as the coordinating element is replaced by an analogous compound of the other elements in the 5th group of the periodic table. Thus arsine, phosphine and stibino and bismuthio (7) hemochromogens have been prepared; their application will await trial in those clinical conditions in which the metals of Group V have proved beneficial.

5. Metalloporphyrins and Metallochromogens.—All of the pigments so far mentioned, originating in hemoglobin, are protoporphyrin derivatives. Except in infinitesimal quantities, normally, or in certain pathologic states occurring so infrequently as to rule them out as a practical source, derivatives of other naturally occurring porphyrins such as those of deuteroporphyrin, cannot be processed directly from blood. This does not mean that the deuteroporphyrins, the mesoporphyrins and aetoporphyrins from each of which an entire chain of ferrihemes, ferridehemes, ferrihemochromogens and ferridehemochromogens as well as their corresponding transitional element porphyrin derivatives may be prepared, are without pharmaceutic interest or possibilities. Their prosecution must await that liberal source of basic material such as exists, for the protoporphyrins, in blood. While the utilization of human erythrocytes is the subject of this report, sources of other porphyrins are crying for exploitation. The problem of what to do with other waste porphyrins is not unrelated to the efficient employment of waste human corpuscles and it is expected that the presently projected study will give impetus to a practical utilization of that storehouse of porphyrin represented by chlorophyl.

Practically all of the porphyrins which have been described as occurring naturally form metallic derivatives with certain of the transitional elements: metalloporphyrins, in which the transitional metal occupies the same position in the molecule as iron in ferriheme. Such metalloporphyrins may occur in nature (turacin; which is a copper porphyrin) or are readily synthesized. The physiology and pharmacology of these derivatives are open fields. For instance, it is not unlikely that they are the mediators for the utilization of certain trace elements (copper, nickel and cobalt) important in mammalian nutrition. Those metalloporphyrins in which the metal has chemical characteristics in common with iron yield a series of metallohemochromogens analogous to and coextensive in range with the large number of potentially active ferrohemochromogens already described. Their pharmacologic prosecution has not as yet been attempted; it awaits only the interest and time of the pharmaceutic investigator.

It can hardly be questioned that the investigations which have been and are being carried on throughout the country with the by-products of blood banking will have important impact upon the pharmaceutics of the future.

SUMMARY

A scheme for the utilization of corpuscular residue from the processing of human blood plasma is presented, the products that can be prepared from it are enumerated and their possible therapeutic applications are discussed.

In addition to the species specific proteins whose applications in therapeutics make their preparation from human erythrocytes a desirable procedure, the preparation of other blood pigment derivatives from any mammalian source as well as prophyrin derivatives from extra hematic sources, are outlined.

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Barbituric Acid Derivatives. Pharmacological Relationship of a Series of Butenyl-Alkyl Barbituric and Thiobarbituric Acid Compounds*

By E. E. Swanson, W. E. Fry, and O. W. Reagan

In a previous communication (1), it was observed that there is an obvious relationship between the pharmacological action and the chemical structure of certain barbituric acid derivatives. In the substitution of primary or secondary alkyl groups in one of the 5-5 positions, with an increasing number of C-atoms in the alkyl chain, both the minimal anesthetic dose (M. A. D.) and the minimal lethal dose (M. L. D.) grow relatively smaller, but when the alkyl radical is longer than 5 C-atoms, the amount required to produce anesthesia or death in rats again increases. Furthermore, as the alkyl chain becomes longer, the therapeutic index or ratio between the M. L. D. and M. A. D. gradually appears to be greater. In general, the duration of action shows similar features; that is, it is shorter when the alkyl group becomes lengthened. In the normal alkyl-substituted derivatives, the critical compound is the one that possesses 6 C-atoms in one of the 5-5 positions; in the secondary alkyl-substituted derivatives, the critical compound is the one having 7 C-atoms in one of the 5-5 positions—beyond either of which the duration of action begins to increase. The substitution of a methyl, ethyl or allyl radical on one of the nitrogens (nitrogen alkyl-substituted barbituric acid derivatives) (2); or the substitution of a methallyl (2-methyl-allyl) (3) or a crotyl (3-methyl-allyl) (4) on one of the 5-5 positions, or a sulfur atom (5) in place of the oxygen on the 2 C-atom also obviously reduces the duration of action. This shorter duration of action is independent of the amount of drug administered. Recently, it was observed that in a series of primary and secondary alkyl-substituted barbituric and

* Received October 5, 1944, from the Lilly Research Laboratories, Indianapolis, Ind.

TABLE I.—BARBITURIC ACID DERIVATIVES INJECTED INTRAPERITONEALLY IN WHITE RATS

R, R′ Barbituric Acid Derivatives	No. of Rats	AD_{50} ± S. E., Mg./Kg.	Av. Duration of Action of Observed M. A. D., Min.	LD_{50} ± S. E., Mg./Kg.
1-Methyl-allyl-ethyl-	40	103.7 ± 9.4	720	180.0 ± 9.1
2-Methyl-allyl-ethyl-	33	111.1 ± 6.8	360	258.8 ± 19.7
3-Methyl-allyl-ethyl-	40	88.43 ± 6.7	300	229.7 ± 5.1
1-Methyl-allyl-allyl-	55	69.01 ± 2.76	456	135.6 ± 6.23
2-Methyl-allyl-allyl-	33	67.82 ± 6.37	380	166.9 ± 8.9
3-Methyl-allyl-allyl-	...	...	...	...
1-Methyl-allyl-*n*-propyl-	50	76.52 ± 2.66	420	227.5 ± 5.5
2-Methyl-allyl-*n*-propyl-	60	114.7 ± 3.7	300	265.1 ± 7.2
3-Methyl-allyl-*n*-propyl-	60	125.0 ± 4.7	120	321.5 ± 3.5
1-Methyl-allyl-isopropyl-	...	...	...	...
2-Methyl-allyl-isopropyl-	30	94.6 ± 3.5	210	207.2 ± 8.9
3-Methyl-allyl-isopropyl-	54	66.1 ± 3.4	200	168.3 ± 7.5
1-Methyl-allyl-*n*-butyl-	45	110.6 ± 2.0	110	222.1 ± 3.0
2-Methyl-allyl-*n*-butyl-	90	92.3 ± 3.7	90	230.1 ± 9.2
3-Methyl-allyl-*n*-butyl-	64	108.8 ± 3.6	40	305.8 ± 5.3
1-Methyl-allyl-sec-butyl-	...	...	...	...
2-Methyl-allyl-sec-butyl-	18	76.30 ± 3.1	150	184.8 ± 3.40
3-Methyl-allyl-sec-butyl-	54	70.7 ± 1.6	120	204.0 ± 8.6
1-Methyl-allyl-isobutyl-	70	80.65 ± 4.28	162	173.1 ± 3.60
2-Methyl-allyl-isobutyl-	67	92.27 ± 3.95	115	221.9 ± 8.10
3-Methyl-allyl-isobutyl-	55	92.4 ± 3.6	45	285.0 ± 6.9
1-Methyl-allyl-sec-amyl-	...	...	...	...
2-Methyl-allyl-sec-amyl-	123	61.83 ± 1.14	90	129.6 ± 1.3
3-Methyl-allyl-sec-amyl-	51	83.97 ± 3.95	66	189.2 ± 6.1
1-Methyl-allyl-isoamyl-	55	127.6 ± 7.5	96	289.4 ± 6.3
2-Methyl-allyl-isoamyl-	106	100.9 ± 1.9	87	237.3 ± 5.8
3-Methyl-allyl-isoamyl-	36	101.6 ± 3.3	40	177.5 ± 7.3

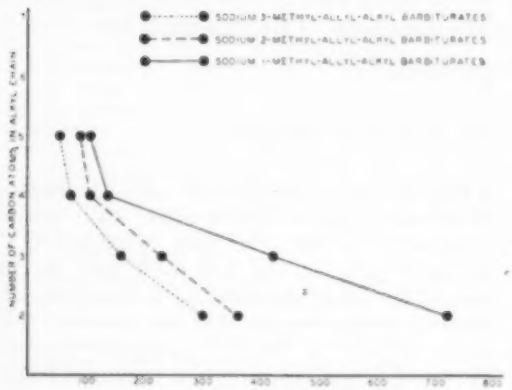
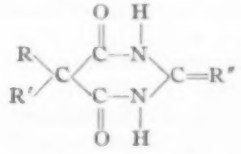


Fig. 1.—Duration of action in minutes.

thiobarbituric acid compounds, the hemolytic time on the sheep's blood increases as the number of C-atoms increases in the substituted alkyl radical (6). In several series of isomeric barbituric acid derivatives, there appeared to be a distinct relationship between the anesthetic and lethal dosage and duration of action, and their chemical structure (7). More recently, in a series of primary and secondary alkyl-substituted barbituric acid thiobarbituric acid compounds, as the number of C-atoms increases in the substituted alkyl chain, the inhibition or sedative action increases on the isolated intestine of rabbits and frogs and the isolated uterus of guinea pigs. The same degree of depression was observed in the perfused frog's heart (8).

EXPERIMENTAL

The present investigation is concerned with the study of a series of butenyl-alkyl barbituric and thiobarbituric acid derivatives, synthesized by Shonle and his associates, of our organic chemical department, with the general formula:



wherein R may be alkyl radicals with 2 to 5 C-atoms, R′, 1-methyl-allyl, 2-methyl-allyl (methallyl) or 3-methyl-allyl (crotyl); and R″, a sulfur atom in place of the oxygen.

Albino rats weighing 80 to 115 Gm. (average 96 Gm.) and New Zealand rabbits weighing 1500 to 2250 Gm. (average 1800 Gm.) were used in this study. Solutions of the sodium salts of the com-

TABLE II.—THIOBARBITURIC ACID DERIVATIVES

TABLE II.—THIOBARBITURIC ACID DERIVATIVES

R, R' / Thiobarbituric Acid Derivatives	Intraperitoneally: No. of Rats	Intraperitoneally: AD_{50} ± S. E., Mg./Kg.	Intraperitoneally: Av. Duration of Action of Observed M. A. D., Min.	Intraperitoneally: LD_{50} ± S. E., Mg./Kg.	Intravenously: No. of Rabbits	Intravenously: AD_{50} ± S. E., Mg./Kg.	Intravenously: Av. Duration of Action of Observed M. A. D., Min.	Intravenously: LD_{50} ± S. E., Mg./Kg.
1-Methyl-allyl-ethyl-	45	124.4 ± 7.58	660[a]	141.8 ± 6.38	20	37.14 ± 2.26	60[a]	59.12 ± 1.85
2-Methyl-allyl-ethyl-	..	.....	....	.....	..	.....	....	.....
3-Methyl-allyl-ethyl-	46	98.15 ± 3.24	672[a]	123.1 ± 5.4	19	26.9 ± 1.97	62[a]	52.57 ± 2.07
1-Methyl-allyl-allyl-	35	104.8 ± 3.14	780[a]	126.3 ± 3.79	35	31.22 ± 1.31	92[a]	58.12 ± 2.98
2-Methyl-allyl-allyl-	..	.....	....	.....	..	.....	....	.....
3-Methyl-allyl-allyl-	35	89.54 ± 5.46	480[a]	172.2 ± 9.30	19	None	[b]	72.86 ± 2.75
1-Methyl-allyl-*n*-propyl-	45	110.0 ± 3.77	660[a]	206.0 ± 6.18	22	37.81 ± 2.14	96[a]	72.85 ± 2.82
2-Methyl-allyl-*n*-propyl-	21	182.5 ± 12.2	900[b]	351.4 ± 16.9	..	.....	....	.....
3-Methyl-allyl-*n*-propyl-	50	226.2 ± 9.5	516[a]	461.9 ± 13.86	75	44.18 ± 2.96	37[a]	121.1 ± 3.90
1-Methyl-allyl-isopropyl-	..	.....	....	.....	..	.....	....	.....
2-Methyl-allyl-isopropyl-	..	.....	....	.....	..	.....	....	.....
3-Methyl-allyl-isopropyl-	60	None	[b]	67.32 ± 2.36	34	22.13 ± 1.48	107[a]	33.36 ± 1.78
1-Methyl-allyl-*n*-butyl-	55	121.8 ± 3.17	684	253.9 ± 6.35	47	27.38 ± 1.94	90	57.24 ± 3.39
2-Methyl-allyl-*n*-butyl-	18	232.5 ± 13.4	600[b]	383.9 ± 15.4	..	.....	....	.....
3-Methyl-allyl-*n*-butyl-	40	None	[b]	398.3 ± 31.27	15	None	[b]	89.66 ± 2.74
1-Methyl-allyl-sec-butyl-	..	.....	....	.....	..	.....	....	.....
2-Methyl-allyl-sec-butyl-	36	None	[b]	96.49 ± 4.05	..	.....	....	.....
3-Methyl-allyl-sec-butyl-	50	124.9 ± 3.4	300[d]	261.0 ± 10.6	50	21.25 ± 1.31	37[d]	52.79 ± 2.36
1-Methyl-allyl-isobutyl-	50	None	[c]	92.04 ± 3.90	40	None	[c]	52.67 ± 2.89
2-Methyl-allyl-isobutyl-	..	.....	....	.....	..	.....	....	.....
3-Methyl-allyl-isobutyl-	40	None	[b]	252.3 ± 6.73	23	61.87 ± 1.81	104[a]	114.5 ± 5.5
1-Methyl-allyl-*n*-amyl-	42	364.5 ± 16.4	180[a]	623.0 ± 16.87	19	99.88 ± 5.64	70[a]	156.6 ± 12.2
2-Methyl-allyl-*n*-amyl-	..	.....	....	.....	..	.....	....	.....
3-Methyl-allyl-*n*-amyl-	..	.....	....	.....	..	.....	....	.....
1-Methyl-allyl-sec-amyl-	..	.....	....	.....	..	.....	....	.....
2-Methyl-allyl-sec-amyl-	40	None	[b]	93.24 ± 4.80	..	.....	....	.....
3-Methyl-allyl-sec-amyl-	55	154.6 ± 4.64	504[e]	341.1 ± 21.91	21	25.86 ± 1.85	30[e]	49.26 ± 2.83
1-Methyl-allyl-isoamyl-	42	None	[b]	444.4 ± 15.6	22	None	[b]	102.9 ± 12.5
2-Methyl-allyl-isoamyl-	..	.....	....	.....	..	.....	....	.....
3-Methyl-allyl-isoamyl-	..	.....	....	.....	8	None	[b]	152.4 ± 3.6

[a] Preanesthetic convulsions.
[b] Pre- and post-anesthetic convulsions.
[c] Severe convulsions without anesthesia.
[d] Abdominal spasms (hiccups).
[e] Tremors.

pounds were injected intraperitoneally in rats and intravenously in rabbits. The minimal anesthetic dose (M. A. D.), duration of action and the minimal lethal dose (M. L. D.) were determined by using five or more animals for each dose level. From these data, the median anesthetic dose (AD_{50} ± S. E.) and the median lethal dose (LD_{50} ± S. E.) were computed according to the formula of Bliss (9). During the tests the animals were kept in a constant temperature room (29.4° to 32.2° C.).

In general, the duration of action of the observed minimal lethal doses decreases as the number of C-atoms increases from 2 to 5 in the substituted alkyl chain (position R) of the butenyl-alkyl barbiturates, as shown in Table I and Fig. 1. The amount (mg. per Kg.) required to produce anesthesia is independent of the duration of action. Furthermore, in the isomeric butenyl-alkyl substituted compounds (where R′ may be 1-methyl-allyl, 2-methyl-allyl or 3-methyl-allyl) as the methyl radical changes from position 1, 2 or 3 in the alkyl chain, the duration of action becomes shorter. Thus, the 1-methyl-allyl-alkyl substituted barbituric acid derivatives have the longest and the 3-methyl-allyl-alkyl barbituric acid compounds, the shortest, duration of action.

In view of the fact that "Sodium Amytal" (Sodium Iso-amyl Ethyl Barbiturate, Lilly) prevented recurrence of convulsions but failed to save the animals receiving lethal doses of 1,3-dimethyl-butyl-ethyl barbituric acid (10), similar experiments were made with the convulsant, 1-methyl-allyl-isobutyl thiobarbituric acid. As an antidote, its isomer, 1-methyl-allyl-*n*-butyl thiobarbituric acid (a thio-derivative with hypnotic and anesthetic properties) was used in place of "Sodium Amytal." As in previous experiments, rabbits were used again. It was observed that single doses (20 to 30 mg. per Kg.) of the anesthetic isomer 1-methyl-allyl-*n*-butyl thio-compound promptly prevented recurrence of convulsions, but failed to save the animals (rabbits) receiving lethal doses (60 mg. per Kg.) of its convulsant isomer, 1-methyl-allyl-isobutyl thiobarbituric acid. In each case, the convulsant thio-derivative was injected by vein, and, after onset of convulsions, was then followed by its hypnotic isomer (1-methyl-allyl-*n*-butyl thiobarbituric acid) by the same route of administration. Thus, to a certain extent, there exists a true antagonism between the two isomeric thiobarbiturates.

TABLE III.—METHYL-ALLYL-BUTYL THIOBARBITURIC ACID ISOMERS INJECTED INTRAPERITONEALLY IN WHITE RATS

R, R′ Thiobarbituric Acid Isomers	AD_{50} ± S. E., Mg./Kg.	Av. Duration of Action of Observed M. A. D., Min.	LD_{50} ± S. E., Mg./Kg.
1-Methyl-allyl-*n*-butyl-	27.38 ± 1.91	90	57.24 ± 3.39
1-Methyl-allyl-*sec*-butyl-	...	...	...
1-Methyl-allyl-isobutyl-	None	[c]	92.04 ± 3.90
2-Methyl-allyl-*n*-butyl-	232.5 ± 13.4	600[a]	383.9 ± 15.4
2-Methyl-allyl-*sec*-butyl-	None	[b]	96.49 ± 4.05
2-Methyl-allyl-isobutyl-	...	...	...
3-Methyl-allyl-*n*-butyl-	None	[b]	398.3 ± 31.27
3-Methyl-allyl-*sec*-butyl-	124.9 ± 3.40	324[d]	261.0 ± 10.6
3-Methyl-allyl-isobutyl-	None	[b]	252.3 ± 6.73

[a] Preanesthetic convulsions.
[b] Convulsions without anesthesia.
[c] Severe convulsions without anesthesia.
[d] Abdominal spasms (hiccups).

In the twenty butenyl-alkyl thiobarbituric acids studied, as shown in Table II, the duration of action also shows a general tendency to become shorter as the number of C-atoms increases in the substituted alkyl chain. Unlike that of the butenyl-alkyl barbiturates (Table I and Fig. 1), most of the butenyl-alkyl thio-derivatives produce either pre- or post-anesthetic tremors, or convulsions, or severe convulsions without anesthesia, or abdominal spasms, when administered intraperitoneally in rats or intravenously in rabbits. For example, as shown in Table III, of the seven isomeric methyl-allyl-butyl thio-compounds tested, six show convulsions or abdominal spasms. One of these isomers, 1-methyl-allyl-isobutyl thiobarbituric acid, produces severe convulsions without hypnosis or anesthesia, similar in type to 1,3-dimethyl-butyl-ethyl barbituric acid, which was extensively studied by Swanson and Chen (10). The convulsions, particularly in rabbits, are violent and tonic in type. The head and forelegs are bent forward and the hind limbs extended backward. The posture of the animals is therefore one of emprosthotonos. The seat of convulsions is probably in the spinal cord, for decerebration or destruction of the medulla does not prevent the occurrence of convulsions, while pithing results in cessation of the spasms.

In a previous report (11), evidence was presented that a convulsant drug, such as picrotoxin or coriamyrtin, following a hypnotic barbiturate has a detoxifying effect; that is, it will wake up animals during sleep and will save their lives from 1.75 minimal lethal doses of "Sodium Amytal." A condition of convulsions could be produced by injecting the convulsant 1-methyl-allyl-isobutyl thiobarbituric acid, following 1 minimal lethal dose (70 mg. per Kg.) of its hypnotic isomer, 1-methyl-allyl-*n*-butyl thiobarbituric acid, given by vein to rabbits; however, no recoveries were observed. In fact, it perhaps hastened death, for minimal lethal doses of the hypnotic (1-methyl-allyl-*n*-butyl thio-) followed by 20 to 30 mg. of its convulsive isomer (1-methyl-allyl-isobutyl thio-) caused death in a shorter time than when single minimal lethal doses of the hypnotic isomer were injected alone. In this respect, a synergistic toxic action is apparently present between the two isomeric thiobarbituric acid derivatives.

In view of the fact that 1,3-dimethyl-butyl-ethyl barbituric acid is a depressant to cold-blooded animals (frogs and toads) and a convulsant to warm-blooded animals (mice, rats, guinea pigs, rabbits,

cats, dogs, and monkeys) (10), similar experiments were made in frogs with the convulsant 1-methyl-alkyl-isobutyl thiobarbituric acid. It was observed that like sodium 1,3-dimethyl-butyl-ethyl barbiturate, the thiobarbiturate (sodium 1-methyl-allyl-isobutyl thiobarbiturate) exhibited marked depression and later anesthesia. Thus, apparently this butenyl thio-derivative is also stimulating to warm-blooded animals and depressing to cold-blooded animals.

CONCLUSIONS

1. A series of butenyl-alkyl barbituric and thiobarbituric acid derivatives have been studied.

2. As the number of C-atoms increases in the substituted alkyl chain, the duration of action decreases.

3. Furthermore, in the isomeric compounds (where R′ may be 1-methyl-allyl, 2-methyl-allyl or 3-methyl-allyl) as the methyl radical changes from position 1, 2 or 3 in the alkyl chain, the duration of action becomes shorter.

4. Many of the butenyl-alkyl thiobarbiturates are stimulating or convulsive in action, particularly, 1-methyl-allyl-isobutyl thiobarbituric acid, which is a severe convulsant to warm-blooded animals and a depressant to cold-blooded animals.

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Preliminary Report of a New Gelatin Product—"Sulfagel"*

By H. L. Flack, D. A. Clarke, and L. F. Tice†

Prior to the Boston Cocoanut Grove fire, a new type of mechanical treatment for burns had been conceived, apparently by two separate groups working individually. The Department of Surgery of The New York Hospital and the Department of Surgery of Johns Hopkins Hospital were following similar lines of research. Apparently instigated by Pickrell's reported work on solution of sulfadiazine in triethanolamine for the treatment of burns (1) the basic idea developed for the use of a prefabricated film for burn therapy. Pickrell's sulfadiazine solution had a series of disadvantages which might be overcome. The solution, being aqueous, dried too slowly. Also, because the film formed is not a protein precipitation resulting from reaction with the surface tissues but rather an eschar formed by the vehicle, the eschar-formation was too slow in developing. To a somewhat similar solution of sulfadiazine, methyl cellulose was added to improve the viscosity of the preparation and to present a substance which would readily form a film. From this last step apparently grew the prefabricated film (2). Employing methyl cellulose, a sulfonamide, triethanolamine, sorbitol and a volatile solvent, a sulfonamide film was prepared and was being investigated clinically at the time of the Cocoanut Grove fire. Some publicity was given the film on that occasion.

The basic idea seemed sound and especially applicable at the battle front, but this film had been found to have a certain number of technical disadvantages, a major one of which is that intimate contact be-

* Received Sept. 5, 1944, from the Dept. of Pharmacy, The New York Hospital, New York; and from the Philadelphia College of Pharmacy and Science, Philadelphia.

† We wish to express appreciation for generous supplies of gelatin and other materials used in this study supplied by the Edible Gelatin Manufacturers' Research Society of America. We are grateful for the cooperation and assistance given by the Department of Surgery of The New York Hospital and especially by Dr. James A. Dingwall III, who was responsible for the clinical work involved.

tween the film and the cracks and crevices of the skin or a burned area cannot be assured; infection has been observed at the site of these air pockets.

A review of the current opinions on burn therapy was given after the Boston fire at a Therapy Conference at The New York Hospital. Observations on the use of a "Sulfafilm" were included and the shortcomings were explained. The character of these adverse factors seemed to present possibilities of elimination by the use of the general idea but with physical properties so modified as to overcome the difficulties.

Because of the remarkable success obtained in the Varicose Vein Clinic and Minor Surgery Clinic of The New York Hospital in the treatment of varicose ulcerations and other old ulcerations of the lower extremities with the Unna Boot,[1] this type of approach was suggested; i.e., a substance which could be applied in a semiliquid state but which would rapidly set into a firm bandage, would follow the contours of the treated area and would penetrate into small cracks and crevices by virtue of its liquid state prior to the formation of a film.

EXPERIMENTAL

A first attempt found impracticable was the simple substitution of sulfanilamide for the zinc oxide in Unna's Gelatin Paste.[1]

Formula No. 1:

Sodium Sulfadiazine	90 Gm.
Pharmagel B[2]	90 Gm.
Glycerin	120 Gm.
A-3 Water[3]	90 Gm.

This combination was used clinically on a patient with a first and second degree burn of the arm and hand. The area had been treated for two days with a "Sulfafilm." The film separated from the wound when fluid collected beneath it. Four days after the application of the above product epithelization appeared normal, the wound was in good condition and continued to heal without further incident. The preparation, necessarily, required the application of heat prior to its use. When sufficiently liquid for application to the burned area, the gel was too warm for comfort although this was a fleeting experience.

The formula and various modifications using the same ingredients, due to the low alkalinity of Pharmagel B plus an autoclaving procedure, precipitates free sulfadiazine. A solution in distilled water of sodium sulfadiazine in the concentration employed had a *p*H reaction of 10.0. The *p*H of the product was 7.5, and it is felt this is the main explanation for the sulfadiazine precipitation. The precipitate did not occur immediately, but was present after autoclaving.

Since speed of drying was a criterion and the formula given was slow, glycerin was eliminated subsequently to hasten drying and setting time.

Samples of the film were inoculated with *Cl. tetani* and *Cl. welchii* to observe its effect on these organisms. No growth occurred; however, subcultures were not made to determine whether this was simply a bacteriostatic action or bactericidal.

To a preparation containing Sodium Sulfadiazine, Pharmagel B and A-3 Water, *N*/10 NaOH was added to obtain a *p*H of 9.1. On standing after autoclaving, no precipitate formed.

Formula No. 2:

Sodium Sulfadiazine	45 Gm.
Pharmagel B	60 Gm.
A-3 Water	75 Gm.
N/10 NaOH to *p*H 9.1	

The preparation was autoclaved at 10 lb. for twenty minutes and then placed in sterile containers. The finished product was semiliquid at room temperature (not requiring heat prior to application). This was applied to an extensive facial burn where the skin had been denuded. It was applied in this instance because a "Sulfafilm" would not follow the many curved contours of the face. However, serous fluid began to form beneath the film before the gel set and the complete dressing was liquefied and had to be replaced with other measures.

This experience suggested the need for a supporting, nonsoluble film to cover the "Sulfagel." Cellophane was employed in sheets and sealed to intact skin with flexible collodion. Later flexible collodion was replaced with collodion because of better drying. The technique was again changed by the use of Johnson and Johnson's "Duo" Liquid Adhesive. At the time of writing, it is felt that a liquid adhesive made of alcohol, ether, castor oil and polyvinyl butyral resin is more desirable as a sealing agent (3).

Buffered Sulfanilamide (Wallace and Tiernan) was tried in the base formula but was found to produce a poorer product than with plain sulfanilamide which, in turn, was not found satisfactory.

Sulfadiazine, because of slower absorption by the tissues and also because it improved the viscosity of the gel, was incorporated according to the following formula:

Formula No. 3:

Sodium Sulfadiazine	20 Gm.
Sulfadiazine	40 Gm.
Pharmagel B	45 Gm.
A-3 Water	90 Gm.

The preparation was autoclaved at 5 lb. for thirty minutes and was opaque as a result of the suspended, free sulfadiazine.

On a dry surface, this gel formed a tough, elastic film. Slight warming on a water bath to body temperature made it applicable. The film dried quickly and gave good clinical results in comparison with "Sulfafilm" (4). However, when dry after

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[1] Unna's Boot technique employs Unna's Gelatin Paste impregnated into bandaging. Various modifications are employed. Unna's Gelatin Paste as employed at The New York Hospital is Pharmagel A 14%, Zinc Oxide 22%, Glycerin 25% and A-3 Water *q. s. ad* 100%.

[2] Supplied by the Pharmagel Corporation, 55 W. 42nd St., New York City.

[3] A-3 Water is distilled water containing 0.52 Gm. methyl *p*-hydroxybenzoate and 0.28 Gm. propyl *p*-hydroxybenzoate per 1000 cc. used to prevent mold growth, etc., in the gelatin.

being applied to burned areas, the film was found to require some supportive measures which will be described.

One of us (H. L. F.) had experimental burns performed to examine formula No. 4.

Formula No. 4:

Sodium Sulfadiazine	25 Gm.
Pharmagel B	50 Gm.
Sulfadiazine	45 Gm.
A-3 Water	100 Gm.

This preparation was autoclaved at 5 lb. for thirty minutes and placed in sterile containers.

Two burned areas were prepared, second degree in depth, one on either leg and involving the same area of surface (4). On the next day the burns were cleaned and dressed, one with "Sulfagel" (Formula No. 4) above and the other with "Sulfafilm."[4] "Sulfagel" was warmed to body temperature and applied to the cleaned area, then covered with several coats of collodion (no cellophane required). "Sulfagel" is transparent when applied. This factor is an advantage in observing progress of the area being treated. "Sulfafilm" was applied by pressing onto the cleaned area, covering with gauze pads and fastening with bandage. The day after its application the "Sulfagel" was turgid and distended with serous exudate and this suggested the "pressure bandage" feature which appears to be a desirable condition. Owens (5) states that a burn is an open, contaminated wound and that the logical treatment consists of (*a*) conversion into a surgically clean wound and (*b*) application of a dressing which adequately protects the wound, directs counter pressure to the underlying tissues and establishes satisfactory drainage from the wound into the body of the dressing. It is our impression that this bandage, either when applied without the formation of exudate pressure or in the state of turgor as noted here, obtains these criteria. On the fourth day the film broke when additional collodion was applied. By this addition, temporary solution of the hardened, retaining collodion resulted and, under pressure, broke. The burn was subsequently treated by daily applications of a tissue extract (4) and on the eighth day both burns were examined. The "Sulfafilm" area had slight granulation and was hemorrhagic. The "Sulfagel" burn was about 50% covered with a good granulation.

A second set of experimental burns was produced, one area on either leg, and treated on the following day; one with "Sulfagel" and one with "Sulfafilm" similar to the above. Two days later the one treated with "Sulfagel" received another application of "Sulfagel." It was then covered with cellophane and sealed with collodion applied to the edges, under which had been placed Compound Tincture of Benzoin. A further protective cellophane film was placed over the dressing and sealed with adhesive to prevent clothing from disturbing the dressing. On the eighth day both burns were examined. The one which was treated with "Sulfagel" was healed, while that treated with "Sulfafilm" was not healed (4).

A third experimental set of contralateral burns was produced, one of which was contaminated by wiping the unsterile areas about the burn with cotton and then daubing this contaminated cotton directly onto the debrided, burned area. This was an attempt to simulate the conditions normally appearing in a burned area. Despite this treatment, the last "Sulfagel" preparation described healed the burn without incident in eight to nine days against eleven days for a similar, but nondebrided burn treated with "Sulfafilm." (In the latter instance, the burn was produced at the same time but was not debrided; i.e., the blister was not removed so that this wound would present the advantages ascribed by some to nondebrided burns.)

[4] Material supplied by Wallace and Tiernan Products, Inc., Belleville, N. J.

DISCUSSION AND SUMMARY

In this study 64 different preparations have been examined employing gelatin and the sulfonamide drugs in an attempt to develop a dressing for burns which would embody the advantages of a preformed film but eliminate some of the disadvantages of this type of film. The various modifications employed have centered about the four base formulas presented in this paper. The last, namely, No. 4, appears to be most desirable. Due to various factors, thorough clinical evaluation was not accomplished; however, in three sets of experimental burns the results were superior to a "Sulfafilm" dressing.

It is recognized that there is considerable opposition to the local use of the sulfonamides as a result of drug fastness or drug allergies developing in some patients. It seems a current opinion that it is better to treat circumscribed areas, where infection is potential, through the blood stream rather than by local application. However, there is to be considered the advantage of obtaining high, local concentrations of sulfonamide drugs by means of local application without elevating the total blood stream concentration by absorption to dangerous levels. In addition, the dangers of drug sensitivity acquired from local application possibly have been overemphasized.

Gelatin appears to us to be a most desirable vehicle for the presentation of many locally effective drugs. The sulfonamides have been examined but, in addition, there are other drugs that might obtain greater utility were they used in a gelatin base. Gelatin approximates the protein composition of the body, it does not produce foreign body reactions when applied to injured tissues, it is readily diffusible and miscible with tissue fluid and carries active agents more readily in this fashion, there being no occlusion.

Pressure bandages are desirable in many injuries. This type of preparation lends its utility in such a fashion to wounds and ulcerative processes without necessarily employing the sulfonamide drugs. A product such as "Sulfagel" is suggested as an appli-

cation to freshly closed surgical incisions, for example, to prevent stitch-abscesses and other surface, postoperative infections.

"Sulfafilm" had previously shown more rapid healing time for burns than had other types of therapy; however, based on limited clinical experience, it appears that "'Sulfagel" gives earlier and better granulation than "Sulfafilm" in burn therapy (4). We would ascribe this, primarily, as due to the difference between a substance which is essentially a foreign body contrasted to a substance which is, after a fashion, similar to animal tissue in its composition. Finally, "Sulfagel," when applied to body surfaces, presents a transparent dressing which possesses certain advantages.

We are not in a position to draw conclusions from this study; however, we perceive some interesting points and have presented this preliminary report with the hope that further work might be stimulated in other groups.

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A Qualitative Test for Dehydroascorbic Acid*

By R. Stuart Tipson†

The United States Pharmacopœia (1) describes three methods of identification and one of assay for ascorbic acid. Although these methods are excellent for reasonably pure samples, they may not be applicable to the qualitative detection of small amounts of the acid in mixtures or in biological materials. There can be no gainsaying the correctness of the results obtained by biological assays for ascorbic acid but the values given by chemical methods of estimation are sometimes to be viewed with caution. Rather elaborate precautions (2) are necessary to ensure that the vitamin shall not become oxidized to dehydroascorbic acid during the preparation of extracts, and if dehydroascorbic acid is already present it is essential that before performing the determination it be reduced, e.g., with hydrogen sulfide, to ascorbic acid. This reduction procedure is not entirely satisfactory because many substances (3) are believed to give rise to an interfering reaction when treated in this manner.

Rather than guard against oxidation, it might be simpler to convert deliberately all the ascorbic acid to dehydroascorbic acid by mild oxidation and then attempt to estimate the latter compound. However, very little work appears to have been done on the determination of dehydroascorbic acid as such, although a procedure has been worked out by Roe and Kuether (4), involving its reaction with 2,4-dinitrophenylhydrazine.

We now find that dehydroascorbic acid reacts at 50° C. with pyrrole, in aqueous trichloroacetic acid, to give an intense blue color with a band at 630–635-mμ. There are three reasons for employing trichloroacetic acid: first, it ensures the precipitation of proteins from biological extracts; second, it provides a *p*H at which dehydroascorbic acid is stable for some time; third, it facilitates the oxidation of ascorbic to dehydroascorbic acid in the presence of activated charcoal (5).

Hence, the color test now described is applied to an aqueous trichloroacetic acid extract or solution of the material under examination; the extract is shaken with activated charcoal and is then filtered. The test is simple, rapid and quite sensitive; a strong blue color is given by an 0.1 per cent solution of dehydroascorbic acid; an 0.01 per cent solution of the acid gives a green color with the *same* amount of pyrrole but if one-tenth the proportion of pyrrole is employed this concentration also yields a blue color readily detected by the naked eye.

In searching for possible interfering sub-

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† The author thanks Drs. Leonard H. Cretcher and J. A. Ruben for their interest in this work.

stances, water-soluble vitamins and other naturally occurring substances were tested but, of the 43 substances so far examined, only alloxan hydrate gives a similar vivid blue color under these conditions. Both substances possess three contiguous carbonyl groups and they have a number of chemical properties in common; the presence of alloxan may, however, be detected by certain specific tests (6) to which ascorbic and dehydroascorbic acid do not respond. Triketohydrindene hydrate (ninhydrin), also possessing three adjacent carbonyl groups, does not give a blue but a green color which rapidly changes to brown and then gray; its presence in biological materials is unlikely.

In its present form, the test has only been employed in a qualitative manner but it appears probable that it should prove adaptable for use as a quantitative colorimetric procedure. It is possible that the method may find application to highly pigmented fruits, since our procedure of shaking with charcoal (to oxidize ascorbic acid to dehydroascorbic acid) usually decolorizes colored extracts.

EXPERIMENTAL

Method of Performing the Pyrrole Test on Pure Ascorbic Acid.—A solution containing ascorbic acid (for example, 100 mg.) and 5 Gm. of trichloroacetic acid per 100 cc. is prepared. Activated charcoal is added to 15 cc. of the solution, the suspension vigorously shaken and filtered through a fluted filter. Two 5-cc. portions of the filtrate are taken, one for use as an unheated control; to each is added 0.02 cc. of pyrrole,[1] the suspension gently agitated for a few seconds until the pyrrole has dissolved, and one portion is then placed in a thermostatically controlled bath at 50° C. A second control, consisting of 5 cc. of 5% trichloroacetic acid plus 0.02 cc. of pyrrole, may be heated at the same time.

For a routine procedure, the solution is heated at 50° C. for five minutes and the color noted; if desired, it may then be cooled in ice. The time required for a blue color to develop with dehydroascorbic acid depends on the concentration (Table I). For very dilute solutions the time of heating may be extended to twenty minutes.

[1] For qualitative tests, 1 drop of pyrrole may be employed.

Notes on the Pyrrole Test.—The solution to be tested should contain approximately 5% of trichloroacetic acid;[2] except as mentioned in the discussion, it is not necessary to boil the distilled water used to prepare the trichloroacetic acid solution. (If the solution to be tested is very dilute in terms of ascorbic acid or its volume is less than 5 cc., the appropriate volume of a freshly prepared 2% solution of pyrrole in 5% trichloroacetic acid should be added instead of 0.02 cc. of pure pyrrole.)

Using the above proportion of pyrrole, a blue precipitate gradually settled during the course of a few hours. However, when one-tenth the amount of pyrrole was employed (i.e. 0.002 cc. of pyrrole per 5 cc. of solution) the formation of this precipitate was considerably delayed, although some settled after standing overnight at room temperature. With the latter amount of pyrrole, 0.01% dehydroascorbic acid gave a blue color displaying a band at 630–635 mμ. The optical densities were measured[3] against a blank (containing 0.02 cc. of pyrrole diluted to 50 cc. with 5% trichloroacetic acid) which had been heated at 50° C. for the same length of time. The determination was made on a Beckmann quartz spectrophotometer,[4] using a tungsten lamp as the light source.

An 0.01% solution of dehydroascorbic acid kept at room temperature during forty-eight hours, and then tested, no longer gives a clear blue color.

Omitting the treatment with charcoal, a very faint blue color was given by ascorbic acid and by alloxantin. If these solutions were shaken with charcoal before performing the test, the rich blue colors caused by dehydroascorbic acid and alloxan, respectively, were observed. Ninhydrin gave the colors described in Table I; an 0.001% solution yielded a clear, pale-green color.

Substances Not Giving the Test.—Since the test might find application to biological materials, substances likely to occur therein were tested; the behavior of some compounds of general interest was also examined. Under the above conditions no blue color was developed with the following pure substances: adenine sulfate, *dl*-α-alanine, allantoin, *p*-aminobenzoic acid, anthraquinone, asparagine, barbital, benzil, caffeine, choline hydrochloride, creatinine, cysteine hydrochloride, cystine, diacetyl, digitonin, ergosterol, *D*-glucose, glutamic acid hydrochloride, glutathione, glycine, glycogen, guanine hydrochloride, *l*-histidine monohydrochloride monohydrate, α-hydrindone, hydroquinone, *i*-inosi-

TABLE I.—COLORS PRODUCED IN THE PYRROLE TEST[a]

Time (in min.), Kept at 50° C.	Fresh Solution in 5% Trichloroacetic Acid			
	Dehydroascorbic Acid	Ninhydrin	Dehydroascorbic Acid	Ninhydrin
	Concentration, 0.1%		Concentration, 0.01%	
1	0	3 + (T, G)	0	1 + (G-Br)
2	2 + (B-G)	5 + (T, G-Br)	0	3 + (Br)
3	3 + (B)	6 + (T, Gr)	1 + (B-G)	3 + (Br)
4	4 + (B)	7 + (T, Gr)	2 + (G)	3 + (Br)
5	5 + (B)	7 + (T, Gr)	2 + (G)	3 + (Br)

[a] Key: B = blue; Br = brown; G = green; Gr = gray; T = turbid; 1+ = faint; 3+ = medium; 5+ = strong; 7+ = very strong.

[2] The use of concentrated trichloroacetic acid is to be avoided since ergosterol gives a blue color with this reagent (7).

[3] This determination was kindly performed by Miss Jacqueline Front.

[4] We are indebted to the Chemistry Department of the University of Pittsburgh for permission to use this instrument.

tol, maleic anhydride, nembutal, nicotinamide, nicotinic acid, *dl*-norleucine, phenanthraquinone, phenobarbital, riboflavin, sodium phenobarbital, thiamine hydrochloride, thiourea, uracil, urea, uric acid and xanthine.

Tests on Vegetable Extracts.—In order to examine the possible utility of the qualitative test, it was applied to extracts of a number of vegetable materials known to contain ascorbic acid; solid samples were extracted by maceration with 1 part of 10% trichloroacetic acid; liquids were treated with one volume of the acid solution. A sample of the filtrate was then shaken with activated charcoal, again filtered, and tested as described above. The following all gave a positive result (blue color): cabbage, carrot, celery, lemon juice, lettuce, mushroom, orange juice, parsnip, spinach and turnip.

DISCUSSION

Rosenberg (8) states that "the accuracy of the determination of vitamin C in extracts of animal origin is somewhat limited. Liver, for example, may give values up to 20% too high." Since it has recently been suggested (9) that liver may contain alloxan (or its reduction products, alloxantin and dialuric acid), it is possible that the presence of the reduced forms might account for the high values obtained in certain vitamin C determinations on liver.

If it is desired to establish the presence or absence of dehydroascorbic acid in a material known to contain ascorbic acid but no alloxan, the precautions necessary to prevent oxidation should, of course, be observed and treatment with charcoal omitted. On the other hand, since dehydroascorbic acid is biologically active, determination by this test of the total dehydroascorbic acid present (after mild oxidation of the ascorbic acid by shaking the solution with activated charcoal) should, in the absence of alloxan, serve to indicate the biological potency; in this case, it is obviously unnecessary to guard against the primary, reversible oxidation of the vitamin.

SUMMARY

1. A simple color test for dehydroascorbic acid is described.

2. The test consists in heating 5 cc. of a solution of dehydroascorbic acid (in 5 per cent aqueous trichloroacetic acid) plus 0.02 cc. of pyrrole during five minutes at 50° C. A strong blue color is developed if 0.1 per cent of dehydroascorbic acid (or alloxan) is present.

3. The procedure may be applied to ascorbic acid by shaking the solution to be tested with activated charcoal, thereby oxidizing it to dehydroascorbic acid.

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The Determination of Iodine in Organic Compounds*

By Paul Block, Jr. and Kenneth L. Waters†

Halogen analyses fall, in general, into four main groups: (*a*) combustion in the presence of a reducing agent; (*b*) wet reductions; (*c*) wet oxidations; (*d*) combustions, usually but not always in the presence of an oxidizing agent. Hans Meyer has described many of these methods in detail (1).

The first of these, developed by Ter Meulen, employs ignition in a stream of hydrogen. It would seem to offer no advantages over its oxidative counterpart, and the use of hydrogen increases the hazards of the method.

The wet reductions most frequently encountered in the literature are of the sodium reduction type (Stepanov) or catalytic hydrogenation. Although both procedures are effective for the quantitative removal of halogen, neither is convenient for multiple analyses. In certain special cases in which it is desired to recover a dehalogenated com-

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pound or to follow the reduction volumetrically, hydrogenation is useful provided the compound does not poison the catalyst.

The methods involving wet oxidation are frequently rapid and accurate with compounds of known types. With the exception of the high-temperature Carius method they lack general applicability. The Carius method is tedious and, for the determination of iodine, has several disadvantages.

The methods of organic analysis which have survived the test of time are those in which the compound being analyzed is completely oxidized; Liebig's carbon-hydrogen determination and the Dumas method for nitrogen are typical examples. Complete combustions have been used for halogen analysis for more than a hundred years. Generally, ignitions have been made in a crucible or tube by mixing the compound with calcium oxide or, less frequently, with magnesium oxide, sodium carbonate, potassium carbonate or the alkali hydroxides. In some cases an oxidizing agent such as potassium nitrate has been added to the ignition mixture. Exact temperature control was seldom employed by the earlier workers. This made the analysis for iodine the least satisfactory of the halogen determinations because of the greater tendency for the iodide to volatilize.

More recently, two forms of dry combustion have found considerable use in the analysis of organic iodine compounds; the Parr bomb and the Pregl methods. In the Pregl method the compound is burned in a stream of oxygen. The method suffers from the absence of a choking plug in the combustion tube, making mandatory extreme care in burning the compound to prevent it from passing through the tube unchanged. Multiple analyses are not feasible, and only micro samples are ordinarily used. The simultaneous determination of ash is an advantage of the method.

The combustion with sodium peroxide in the Parr bomb is advantageous for the more volatile compounds. However, it has many disadvantages, e.g., the instability of sodium peroxide, particularly in damp weather or in the presence of organic liquids; the necessity of preparing the fusion mixture with great care in order to avoid either explosion or incomplete combustion; and the difficulty of removing all traces of peroxide after the combustion in order to determine the iodide volumetrically. (A method for performing this on a micro scale has been described (2).)

Several years ago Johnson and Frederick (3), after extensive study of various reagents which had previously been suggested for ignition mixtures, evolved a combination consisting of manganese dioxide, potassium carbonate and sodium carbonate, which was very satisfactory for the determination of iodine in livestock mineral mixtures. The authors have successfully adapted this method to determination of iodine in organic compounds. Results obtained with this method compare favorably with those obtained by the official assays of the United States Pharmacopœia.

The method offers many advantages: (*a*) using approximately the time and temperature suggested, loss of iodine, even from milligram quantities, is negligible; (*b*) there is no frothing or expansive boiling and overflow; (*c*) the combustion mixture does not fuse, thus eliminating a time-consuming lixiviation of iodide from the melt; (*d*) the number of analyses which may be conducted simultaneously is limited only by the size of the muffle furnace available; (*e*) macro, semimicro or micro samples may be taken without any change in the combustion procedure; (*f*) no special apparatus is needed other than an electric furnace equipped with a pyrometer; (*g*) after filtering the manganese dioxide, the solution is free from reduction products or other interfering substances and the iodide may be determined immediately.

In recent years analysts have recognized the superiority of determining iodide by oxidation to iodate and subsequent reaction of the latter with potassium or sodium iodide, taking advantage of the six atoms of iodine liberated for each iodine atom in the sample. To effect the oxidation, saturated bromine water is the reagent most frequently encountered; its use is described below.

EXPERIMENTAL

Method.—Weigh a sample (0.1–0.2 Gm.) into a porcelain crucible (No. 2 high). Add 17 Gm. of ignition mixture (Note 1) and mix thoroughly. Overlay with a further 6 Gm. of the ignition mixture. (These quantities should be weighed to 0.2 Gm.) Ignite for twenty-five minutes in a muffle furnace preheated to 675–700° C. Remove from the furnace, allow to cool, and empty the contents of the crucible into a 250-cc. beaker. Remove any solid material which adheres to the sides of the crucible and rinse thoroughly with hot water. Crush with a stirring rod any lumps which may have formed. Dilute to about 100 cc. with water and digest for fifteen minutes on the steam bath. Cool, transfer the contents of the beaker to a 500-cc. volumetric flask and make up to the mark. Add an additional

amount of distilled water to compensate for the volume occupied by the manganese dioxide present (Note 2). Mix completely and allow to settle for about five minutes. Filter through a dry filter (Whatman No. 2 or equivalent) into a dry flask, discarding the first 50 cc. of filtrate.

Transfer a 200-cc. aliquot to a 500-cc. Erlenmeyer flask and determine the iodine content as follows: Add an excess of freshly prepared, saturated bromine water (10 cc. is usually sufficient) (Note 3). Add 17 cc. of phosphoric acid prepared by mixing 1 volume of 85% phosphoric acid, U. S. P., with 1 volume of water measured separately. This addition should be made slowly with swirling, and preferably from a burette. Boil the solution until practically colorless to remove excess bromine (Note 4). Cool and add 5 cc. of 5% aqueous phenol. Wash down the sides of the flask and allow the solution to stand for five minutes. Add 5 cc. of 10% sodium or potassium iodide and titrate immediately with 0.1 *N* thiosulfate solution, using starch indicator (Note 5).

Note 1.—The ignition mixture consists of 106 parts of sodium carbonate (anhydrous), 138 parts of potassium carbonate (anhydrous) and 113 parts of manganese dioxide. The ingredients should be in the form of fine powders but should not be ground together. A blank should be run for each lot of reagents used. Manganese dioxide may be encountered which gives a blank greater than 0.2 cc. of 0.01 *N* thiosulfate or bestows a yellow color on the filtrate. This does not interfere appreciably in the titration with 0.1 *N* thiosulfate, but is objectionable with more dilute solutions. If such a manganese dioxide is encountered it is well to purify the material by ignition with alkali carbonate, adding water and boiling for a few minutes, then washing free of alkali and drying.

Note 2.—Generally, the volume occupied by the manganese dioxide is under 2 cc. A correction may be obtained by placing 7.5 Gm. of manganese dioxide in a 500-cc. (or other) volumetric flask and pipetting in exactly 500 cc. of water, the corrected volume being obtained by marking the neck of the flask. The allowance for solid material in a volumetric analysis is not without precedent (4).

Note 3.—For the macro analyses reported in this paper, reagent quality bromine has been used by the authors without purification. If there is a blank of more than 0.2 cc. of whatever strength thiosulfate solution is employed (depending on the size of the sample and aliquot), the cause may be the iodine content of the bromine. The bromine may then be purified, as by the method of Karns and Donaldson (5).

Note 4.—The bromine oxidation described has been found to be general. However, in the routine determinations of compounds of high iodine content where the weight of the sample does not exceed 0.25 Gm., the boiling step can be omitted, provided that care is used in avoiding a large excess of bromine. In this modification, the aliquot is first acidified with the phosphoric acid, and bromine is added slowly until the shade of the solution approaches that of a solution of 250 cc. of water containing 1.5 cc. of saturated bromine water. The brownish color which appears at first must not be confused with the golden yellow which subsequently develops when bromine is in excess. After the addition of bromine, the solution is allowed to stand two to three minutes, and the excess bromine removed by addition of 5 cc. of the phenol solution. In this way the time required to boil and cool the solution is saved.

Note 5.—Solutions of thiosulfate more dilute than 0.1 *N* may be used by taking aliquots smaller than 200 cc. A 50-cc. aliquot then requires 4.5 cc. of the 1 + 1 phosphoric acid, and 100 cc. requires 8 cc. of the acid. These amounts should be measured carefully as they are designed to bring the acidity close to *p*H 2.5. When 0.01 *N* or 0.005 *N* thiosulfate is employed, as for micro samples, the starch indicator must be specially prepared. For this purpose, arrowroot starch is treated with a solution of 1 volume of hydrochloric acid in 3 volumes of water. The suspension is allowed to remain at 20° C. for twenty-four hours, filtered, washed carefully with water containing a few drops of ammonia, and three times more with water; it is then dried in a desiccator. A 0.5% solution is made by adding the starch, triturated with a little water, to boiling water and allowing the ebullition to proceed for one minute. The cooled solution may be preserved by the addition of 20% of sodium chloride, although for careful work it should be freshly prepared. Ten cubic centimeters of this solution is used for 200 cc. of solution being titrated.

TABLE I.—RESULTS OF DETERMINING IODINE IN ORGANIC COMPOUNDS BY VARIOUS METHODS

Compound	MnO_2 Method	U. S. P. XII Assay	Theory
Tetraiodophenolphthalein, Sodium U. S. P. (free acid)	60.7 (5)[a]	60.8 (4)	...
Chiniofon, U. S. P.	27.8 (2)	27.6 (4)	...
Calcium Iodobehenate, U. S. P.	25.7 (2)	25.3 (3)	...
Thymol Iodide, U. S. P.	43.5 (2)	42.7 (2)	...
Iodoform (N. F.)	96.1 (2)	96.2 (3) (N. F. assay)	...
$C_7H_5OI_2$[b]	78.4 (9)	...	78.4
C_3HO_3I[c]	62.5 (2)	...	62.8
$C_{14}H_{10}O_3I_2$[d]	52.8	...	52.9
$C_{13}H_9O_5NSI_2$[e]	46.5	...	46.6

[a] Numbers in parentheses refer to number of analyses made.

[b] Triiodophenyl methyl ether recrystallized to a constant m. p. (98–99° C.).

[c] Glycerol iodohydrin recrystallized to constant m. p.

[d] Diiodomethoxyphenoxybenzaldehyde recrystallized to constant m. p.

[e] Tosyl ester of diiodonitrophenol recrystallized to constant m. p.

DISCUSSION

Results of analyses, by this method, on four United States Pharmacopœia compounds, one National Formulary compound and four other organic iodide compounds are shown in Table I. In all cases duplicates checked within four parts per thousand and in most cases within two parts per thousand. The maximum variation encountered was with tetraiodophenolphthalein. The results of five analyses were 60.3, 60.5, 60.7, 60.8 and 61.0% iodine. The loss of iodine during ignition appears to be negligible, as evidenced by the very excellent agreement with official methods. Good results were also obtained on glycerol iodohydrin, a low-melting

compound. However, when the analysis of an organic liquid, iodobenzene, was tried there was apparently loss of iodine during ignition as results were about 4% low.

The exact role of the manganese dioxide is not known. At temperatures high enough to effect complete destruction of the compound in the stated time, omission of the manganese dioxide from the combustion gives erratic and low results. This does not seem to be true when a large amount of organic matter is present, e.g., when large samples of thyroid gland, or feeds containing only a small amount of iodine, are analyzed. The stabilizing action of organic matter has been noted by Johnson and Frederick (3).

Low results were also obtained when sodium carbonate was substituted for the potassium carbonate in the ignition mixture. The reverse of this, that is, omitting the sodium carbonate in the mixture, offered more promise, but has not yet proved itself as reliable as the mixture of carbonates suggested.

CONCLUSIONS

1. The method of Johnson and Frederick has been shown to be applicable to the analysis of pure organic compounds.
2. The procedure presented shows promise of being general for all but the most volatile organic iodine compounds.

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Book Reviews

Fats and Oils, An Outline of Their Chemistry and Technology, by H. G. KIRSCHENBAUER. Reinhold Publishing Company, New York, 1944. 154 pp. 15 x 23 cm. Price, $2.75.

A book in outline, while performing a very useful service, is always open to criticism because of the relative incompleteness of many of its sections. However, when such a book is measured in terms of its function it often proves to be worthy. Such is the case with this book on fats and oils. Intended to furnish general background material for initiates, it provides brief, concise information on the fundamentals of fat chemistry and technology.

The opening section of this handy little volume is concerned with the constituents of fats. One of the convenient portions of this section is a tabular arrangement showing the properties of the fatty acids. Indeed the generous sprinkling of tabular data throughout the book contributes much to its usefulness.

The chapter on analytical methods will appeal to the pharmaceutical chemist. This section is, however, of necessity short and concerned almost entirely with a brief description of standard methods and is not a critique.

Sixty pages of *Fats and Oils* are given over to a discussion of the technology of fats. Modern and some older methods of fat technology, especially soap making, are outlined here briefly and made clear by the use of diagrams and flow sheets.

The appendix, which includes important historical dates, the fatty acid composition of the most significant fats of commerce, the analytical character of fats and waxes, and a bibliography, is an appealing feature.—MELVIN W. GREEN.

Procedures in Experimental Physics, by JOHN STRONG. Prentice-Hall, Inc., New York, 1944. x + 642 pp. 15 x 23 cm. Price, $5.00.

At first glance at the title, this book might not appear to be of interest to the pharmacist. However, the researcher in pharmacy will find it quite useful occasionally.

Procedures in Experimental Physics is intended to be a guide to the instrumentation of physics. Since many instruments of physics are used in laboratory procedures in pharmacy, this will be found a useful guide to the making and repairing of these instruments.

To review a book of this type is difficult for one who has had so little experience in instrumentation. A cataloguing of some of the more pertinent operations described might help the reader to determine its scope. Fundamental operations in glass blowing, work at the optical bench, the technique of high vacuum, the use of fused quartz, electrometers and electroscopes, Geiger counters, the measurement of radiant energy, photoelectric cells, notes on research materials such as alloys, individual metals, soapstone, etc., instrument design, molding and casting—all of these topics and many more are discussed and amply illustrated. This is a convenient book for the shelves of a research laboratory.—MELVIN W. GREEN.

Identification of Sulfapyrazine through Ultraviolet Fluorescence of 2-Aminopyrazine*

By Harry W. Raybin

Neutral solutions of 2-aminopyrazine (1), the pyrolysis product of sulfapyrazine, have been found to give an intense blue-violet fluorescence in the ultraviolet, most vivid in the 3650 Å. region[1]. The characteristic blue fluorescence is not given by aqueous solutions (filtered) of the following sulfa compounds: sulfapyrazine, sulfadiazine, sulfamethyldiazine, sulfadimethyldiazine, sulfathiazole, succinylsulfathiazole, sulfanilamide, sulfaguanidine and sulfapyridine. Also negative are 2-aminopyrimidine, 2-amino-4-methylpyrimidine and 2-amino-4,6-dimethylpyrimidine. The fluorescence is easily visible in a dilution of one part per million.

Three of the sulfa drugs at present in the U. S. P. XII and the 1944 New and Nonofficial Remedies, i.e., sulfadiazine, sulfapyrazine and sulfamerazine (sulfamethyldiazine) give crystalline pyrolysis products suitable for identification purposes. However, the melting points of sulfadiazine, 250–254° C., and its pyrolysis product 2-aminopyrimidine, 126–127° C. (after recrystallization), and of sulfapyrazine, 252–256° C., and its pyrolysis product 2-aminopyrazine, 120–122° C., are fairly close. Sulfamerazine melts at 235–236° C. and its pyrolysis product 2-amino-4-methylpyrimidine at 159–161° C. (recrystallized).

Sulfadiazine is readily distinguished by the fact that its pyrolysis product, 2-aminopyrimidine, gives a color reaction with resorcinol (2).

The blue fluorescence in the ultraviolet now reported is characteristic for aqueous solutions of 2-aminopyrazine, and thus for sulfapyrazine. The fluorescence is easily visible in a dilution of one part per million.

The test readily lends itself to microtechnique. After performing the melting-point determination on sulfapyrazine, the capillary melting-point tube is kept in the bath at melting-point temperature (254° C.) for three minutes to obtain sufficient 2-aminopyrazine for the test. The capillary, wiped clean of bath fluid, is cut off above the charred lower end, and the upper end ground with 5 cc. distilled water in a mortar. The filtered solution is then observed in a source of ultraviolet in the dark room ($2^1/_2$ watt argon glow lamp is sufficient) (3). A filtered solution of sulfapyrazine serves as a negative control.

SUMMARY

Neutral solutions of 2-aminopyrazine, the pyrolysis product of sulfapyrazine, give a characteristic blue-violet fluorescence in the ultraviolet suitable for identification purposes.

ADDENDUM

After this paper was submitted, there appeared a paper by Weijlard, J., Tishler, M., and Erickson, A. E., on "Some New Aminopyrazines and their Sulfanilamide Derivatives" [*J. Am. Chem. Soc.*, **67**, 802(1945)]. Dr. Weijlard kindly furnished me with some samples (several years old) of the following 2-amino-pyrazines: 5,6-dimethyl; .6-methyl; 5-methyl; 5,6-dimethyl-3-carboxylic acid; 6-methyl-3-carboxylic acid; and 5,6-diphenyl. All except the 2-amino-5,6-diphenyl-pyrazine were found to fluoresce similarly to 2-amino-pyrazine. The corresponding sulfanilamides are still in experimental use only.

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* Received March 16, 1945, from the Chemical Laboratory, Bureau of Food and Drugs, New York City Department of Health.
[1] Acknowledgment is made to E. W. Beggs and A. J. Dusault of the Commercial Engineering Department of the Westinghouse Electric and Manufacturing Co., Lamp Division, Bloomfield, N. J., for some ultraviolet fluorescent measurements.

The Pharmacologic Action of Certain Lycopodium Alkaloids*

By Henry M. Lee and K. K. Chen†

The genus *Lycopodium*, family Lycopodiaceae, or the club mosses, has been of interest in medicine by virtue of the fact that the spores of one of the species, *L. clavatum*, have been used as a dusting powder. It has been known for a long time that some species of *Lycopodium* contain alkaloids. Bödeker (1) first described lycopodine, isolated from *L. complanatum*, to which he assigned the empirical formula, $C_{32}H_{52}N_2O_3$. Later, Arata and Canzoneri (2) reported a poisonous alkaloid obtained from *L. saururus*, which they named pillijanine, with a composition of $C_{15}H_{24}N_2O$. Their work was confirmed by Dominguez (3). Other publications on this subject are those of Orechoff (4) and Muszyński (5). The former worked on the alkaloids of *L. annotinum* and the latter on those of five European species, namely, *L. selago*, *L. annotinum*, *L. inundatum*, *L. complanatum*, and *L. clavatum*. Achmatowicz and Uzieblo (6) characterized three alkaloids of *L. clavatum*. These were lycopodine, $C_{16}H_{25}NO$; clavatine, $C_{16}H_{25}NO_2$; and clavatoxine, $C_{17}H_{27}NO_2$. From an Argentine species, *L. saururus*, Deulofeu and De Langhe (7) succeeded in isolating two new alkaloids, saururine, $C_{10}H_{19}N$, and sauroxine, $C_{17}H_{26}NO_2$.

Pharmacologically, the galenical preparations of the *Lycopodium* species have been studied by Oficjalski (8) and Nikonorow (9). Some information is also available concerning the action of several pure alkaloids. Arata and Canzoneri (2) showed that pillijanine caused violent convulsions and vomiting in dogs. Achmatowicz and Uzieblo (6) reported that lycopodine, clavatine, and clavatoxine stimulated the respiratory center in mammals and paralyzed the central and peripheral nervous system of frogs.

At the National Research Laboratories, Ottawa, Canada, Manske and Marion (10–14) recently made an elaborate study of *Lycopodium* species, particularly, *L. complanatum*, *L. annotinum*, *L. tristachyum* and *L. obscurum*. A total of nineteen alkaloids were successfully isolated, seventeen of which are new. Through the generosity of Dr. Richard H. F. Manske, six of these alkaloids were made available to us for a pharmacologic study. They are lycopodine, complanatine, obscurine, annotinine and two unnamed principles designated L8 and L9. The first three were derived from *L. complanatum*, and the last three from *L. annotinum*. For animal experiments, all six alkaloids were dissolved in equimolecular amounts of hydrochloric acid and diluted to suitable concentrations. In most instances, a 1 per cent solution was employed.

Fig. 1.—Action of Obscurine on Blood Pressure

Cat, female, weight 2.952 Kg., was decerebrated and pithed. Artifical respiration was instituted. All injections were made intravenously. The epinephrine responses were recorded for sake of evaluating the pressor potency of obscurine.

1. Blood Pressure.—An intravenous injection of 5 mg. (total) of lycopodine in etherized cats was followed by a slight, transient elevation of carotid blood pressure. Higher doses, 10–20 mg., caused a depressor effect in either etherized or pithed cats. Complanatine or obscurine, injected by vein in etherized cats, in the dose of 5 mg. similarly raised blood pressure slightly. In pithed cats, obscurine produced a more distinct and sustained rise of blood pressure, as illustrated in Fig. 1. The average intensity of pressor value per mg. of obscurine, determined in 5 pithed cats, was equivalent to 0.4–0.5 γ of epinephrine. The action of annotinine was variable. In one etherized cat, a dose of 5 mg. injected intravenously raised blood pressure to the extent of 30 mm. Hg, but in another cat the same dose was ineffective. Injections of 10–20 mg. in a third cat resulted in a slight rise. A few observations were made with L8 and L9. In general, a dose of 20 mg. of each had a slight pressor action.

2. Respiration.—In all etherized cats, respiratory excursions were recorded by the tambour method. None of the six alkaloids had much influence upon the amplitude or rate of respiration.

3. Isolated Smooth Muscle Organs.—Lycopodine and L8 in the concentration of 1:20,000–1:10,000 augmented the peristaltic movements of isolated rabbit's small intestines, immersed in

* Received July 31, 1944, from the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Ind.

† We are indebted to Messrs. Charles L. Rose, John C. Hanson, Wallace C. Wood and Francis W. Israel, for their invaluable assistance in these experiments.

Locke-Ringer's solution kept at 38° C. Complanatine, obscurine, annotinine and L9 were ineffective in the same concentrations. When tested on isolated rabbit's uterus, immersed in Tyrode's solution maintained at 38° C., lycopodine, complanatine, obscurine and L9 all caused contractions in dilutions of 1:40,000–1:10,000, lycopodine being the most powerful. In the same dilutions, annotinine was ineffective. Lycopodine, L8 and L9 contracted isolated guinea pig's uterus, also with concentrations of 1:40,000–1:10,000. Annotinine was again inactive.

4. Frogs.—Lycopodine in doses of 0.1–0.2 mg. per Gm. of body weight, injected into the ventral lymph sac, caused incoordinate movements leading to complete paralysis. One animal injected with 0.1 mg. of complanatine per Gm. acted similarly. Doses of 0.1 and 0.2 mg. of annotinine per Gm. were without effect, but one of 0.4 mg. per Gm. caused ataxia, while one of 0.6 mg. per Gm. led to complete paralysis. L9 in the dose of 0.2–0.4 mg. per Gm. provoked incoordinate movements much in the same manner as induced by the other alkaloids. No studies were made with obscurine or L8 on account of insufficient material.

5. Experimental Malaria.—Manske and Marion (11) demonstrated that lycopodine upon dehydrogenation yields a mixture of five bases, two of which have been identified as 7-methyl quinoline and 5:7-dimethyl quinoline. It seemed desirable to test lycopodine for any antimalarial activity in view of the present-day interest in quinine substitutes, the latter and pamaquine being also quinoline derivatives. Ducklings were infected by transfusion with *Plasmodium lophurae*. Lycopodine in the doses of 1 and 10 mg. per Kg. per day was injected intravenously for five days. No disappearance of parasitemia occurred. The alkaloid was therefore ineffective.

6. Acute Toxicity.—By intravenous injection in albino mice, the median lethal doses ± standard errors of lycopodine, complanatine, obscurine, annotinine and L9 were determined, as shown in Table I. Complanatine is the most toxic, and annotinine the least toxic, alkaloid. It should be recalled that the action of annotinine is relatively feeble on blood pressure and smooth muscle organs. The activity and toxicity of these alkaloids probably run parallel. Lethal doses of all five alkaloids caused convulsions. Near-lethal doses also produced convulsions, but the animals promptly recovered.

TABLE I.—TOXICITY OF FIVE LYCOPODIUM ALKALOIDS INJECTED INTRAVENOUSLY IN MICE

Alkaloid	Dose, Mg./Kg.	No. Died No. Used	LD_{50} ± S.E., Mg./Kg.
Lycopodine	22.5	0/8	27.58 ± 1.16
	25.0	7/16	
	27.5	5/18	
	30.0	5/8	
	33.0	6/8	
	36.5	6/8	
	40.0	8/8	
Complanatine	12.5	0/8	14.87 ± 0.46
	14.0	4/8	
	16.0	5/8	
	18.0	8/8	
Obscurine	62.0	0/8	99.17 ± 11.29
	70.0	3/8	
	80.0	2/8	
	90.0	3/8	
	100.0	4/8	
Annotinine	90.0	0/8	114.6 ± 2.94
	100.0	2/16	
	110.0	4/16	
	125.0	7/8	
	160.0	8/8	
L9	33.0	0/8	40.28 ± 1.49
	36.5	4/8	
	40.0	4/8	
	45.0	5/8	
	50.0	7/8	
	56.0	8/8	

SUMMARY

1. A preliminary, pharmacologic study has been made with six *Lycopodium* alkaloids: lycopodine, complanatine, obscurine, annotinine, L8 and L9.

2. Appropriate doses of these alkaloids injected intravenously in cats raise arterial blood pressure. Obscurine has the most pronounced pressor action.

3. The above alkaloids have no effect upon the respiration of anesthetized cats when injected intravenously.

4. Lycopodine and L8 augment the peristaltic movements of isolated rabbit's small intestines. Lycopodine, complanatine, obscurine and L9 stimulate the isolated rabbit's uterus. Lycopodine, L8 and L9 contract isolated guinea pig's uterus.

5. Lycopodine, a quinoline derivative, has no antimalarial activity in ducklings.

6. Lycopodine, complanatine, annotinine and L9 in sufficient doses cause muscular incoordination and paralysis following injection into the lymph sac of frogs.

7. The toxicity of lycopodine, complanatine, obscurine, annotinine and L9 has been determined in mice by intravenous injection, complanatine being the most, and annotinine the least, toxic.

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Alkaloids in *Nicotiana attenuata* and *Nicotiana trigonophylla**

By C. Verne Bowen

Marsh, Clawson and Roe (1) in 1927 reported on certain wild tobaccos as stock-poisoning plants. The species investigated were *Nicotiana attenuata* and *N. trigonophylla*. Couch (2) in 1927 reported that *N. attenuata* contained nicotine to the extent of 1.45 per cent in the leaves, 0.48 per cent in the stems, and 0.25 per cent in the roots. In view of the knowledge regarding nornicotine at that time, it was possible that small amounts of this alkaloid may have been present but were not detected by the means used to identify the alkaloidal material.

Marsh, *et al*, (1) in discussing *Nicotiana trigonophylla* stated that "the characteristic picrate is sufficient to identify the alkaloid as nicotine. The quantity of nicotine in the plant was determined, using the silicotungstic method of Shedd, and found to be 0.39 per cent." The appearance of the picrate crystals is not reliable in showing the absence of alkaloids other than nicotine, since it has been observed in this laboratory that the presence of nornicotine up to approximately 20 per cent in a nicotine-nornicotine mixture does not produce picrate crystals appreciably different from those of pure nicotine. Shmuk and Borozdina (3) in 1941 reported nornicotine as the main alkaloid of *N. trigonophylla* with a total alkaloid content of 0.12 per cent and containing "traces of tertiary pyridic alkaloids and, possibly, also of nicotine." And in 1942 Smith and Smith (4) reported *N. trigonophylla* as containing 0.06 per cent of nornicotine and no nicotine. They did not make any analysis of *N. attenuata*.

Recently described methods of analysis (5, 6) now make it possible to determine nicotine and nornicotine readily when present together in plant material. Analyses by one of these methods (5) are given in Table I for samples of *Nicotiana attenuata* from near Twin Falls, Idaho, and *N. trigonophylla* from near Tucson, Ariz. The melting points of the picrates of the mixed alkaloids, both before and after methylation, are given as confirmation of the nornicotine (7). The larger amount of alkaloid in the roots is interesting. The total steam-volatile alkaloids from *N. attenuata* were also analyzed according to Markwood's method (8), giving 1.82 per cent of nicotine and 0.14 per cent of nornicotine.

It should be remembered that the alkaloid content of a plant varies according to the soil foods, plant age, flowering, seed formation and other horticultural practices. Consequently, any one analysis is not representative of all plants of the species.

TABLE I.—ALKALOID CONTENT OF *Nictoiana attenuata* AND *N. trigonophylla*

Species	Portion of Plant	Picrate Melting Point (Cor.), ° C.		Analysis, %		Nornicotine in Total Alkaloid
		Original	Methylated	Nicotine	Nornicotine	
N. attenuata	Aerial	213–225	224–225	1.90	0.14	6.9
N. trigonophylla:						
Sample 1 (4 plants)	Aerial	...	...	None	0.08	100
	Root	180–182	223–224	0.05	0.31	86
Sample 2 (large plant)	Aerial	...	...	Trace	0.25	100
	Root	177–179	224–225	0.05	0.32	87

* Received Oct. 13, 1944, from the Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Md.

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Book Reviews

Handbook of Chemistry. Fifth Edition. Compiled and edited by NORBERT ADOLPH LANGE. Handbook Publishers, Inc. Sandusky, Ohio, 1944. xvi + 1777 pp., Mathematical Appendix 271 pp. 13 x 20 cm. Price, $6.00.

A book which has undergone five revisions during the short period of ten years and is seen on the desk of nearly every pharmaceutical chemist in the United States scarcely needs to be reviewed.

Essentially the same tables found in former editions are to be found in this one. These older tables have been augmented to occupy, with the new tables, 174 additional pages. The table of *Physical Constants of Organic Compounds* has been revised to contain 6507 organic compounds with a corresponding list of 3500 synonyms. The addition of Beilstein references, wherever possible, is a convenient feature of this section.

The tables added for the first time in this edition are: periodic table, flammable liquids, flame temperatures, plastics, fluorescence of chemicals, minerals and gems, and water for industrial use.

This handbook is used very frequently for convenient reference in the Laboratory of the AMERICAN PHARMACEUTICAL ASSOCIATION.—MELVIN W. GREEN.

The Marihuana Problem in the City of New York, by THE MAYOR'S COMMITTEE ON MARIHUANA. The Jaques Cattell Press, Lancaster, 1944. xii + 220 pp. 15 x 23 cm. Price, $2.50.

For over a decade, marihuana has been rumored to play an important role in crime and social decadence. At the insistence of the rumor, Mayor LaGuardia requested the New York Academy of Medicine to make a complete survey of the problem as it applied to the city of New York. The result, which is here reviewed, comprises an excellent broad survey of the social, clinical, pharmacological and chemical implications of this drug.

The sociological phase of the marihuana problem is as fascinating as a detective story. It is of interest to note that, while the use of the drug is widespread in Manhattan and centered in Harlem, its smoking among school children is not very widespread and it is not a serious contributor to juvenile delinquency. The distribution of this drug is not an organized racket as in the case of the more catastrophic morphine market. In the opinion of this deliberating body, marihuana is not a determining factor in the commission of major crimes.

To the reviewer it appears clear that the Committee does not believe the problem is much above the "nuisance" level. The Committee is to be commended for reporting what it finds without resorting to propaganda and, above all, for avoiding hysteria. Much of the press attack against marihuana is apt to intensify the problem.

The clinical study involved in this report is arranged under the following broad headings: medical aspects, psychological aspects, comparison between users and nonusers from the standpoint of mental and physical deterioration, addiction and tolerance, and possible therapeutic applications.

Carefully controlled studies were made on many physiological functions such as hematology, effects on circulation, liver damage, basal metabolism, etc. In general, it was agreed that the positive actions noted are due to forms of central excitation transmitted through the autonomic nervous system and not a direct action on the organs themselves.

The psychological phase of the study indicates quite clearly that mental impairment is concommitant with the taking of marihuana and is roughly proportional to the dose taken. Mental deterioration does not appear to take place, however. Emotionally, persons with limited capacity for affective experience who have difficulty in making social contacts are more likely to resort to the use of the drug in order to overcome these factors at least temporarily.

Morphine and heroin addicts at Riker's Island Penitentiary were studied with a view to using tetrahydrocannabinol as a replacement. While only a total of 56 addicts were studied, the impression was gained that those receiving the tetrahydrocannabinol had less severe withdrawal symptoms. Probably this aspect of the survey deserves further study.

To pharmacists, particularly those who are interested in research, the section of this book dealing with pharmacological aspects will be the most interesting. This review is centered about the chemical and pharmacological identification of the active principles of hemp and an attempt is made to coordinate chemical structure with physiological activity. Of over 65 substances studied, tetrahydrocannabinol was shown to be the most active. A thorough pharmacological study was made on this compound as well as on several others. That the action is a basically central stimulant is indicated by the fact that amphetamine augments the ataxia while amytal has no effect. This study is extraordinarily complete for a survey of this type, and many interesting facts were brought to light. —MELVIN W. GREEN

Statistical Adjustment of Data, by W. EDWARDS DEMING, John Wiley and Sons, New York, 1938. v + 261 pp., 14 x 20.5 cm. Price, $3.50.

Adjustment of data is the central theme expressed in this book. In scope the book is purely supplemental and is intended for the initiated. To the person without a knowledge of the fundamentals of statistical mathematics this text will be of little use. To the specialist, however, it will aid in the solution of many specialized problems in sampling.

The book is divided into five parts. Part A discusses simple adjustments. In Part B more complicated problems are solved by the method of least squares. Parts C and D deal with conditions with and without parameters while Part E deals with special exercises and notes. Examples are carefully selected from forestry, general commerce, the physical sciences and other fields.—M. W. GREEN.